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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Petitte *et al.*	Group Art Unit: 1632
Appln. No.: 09/757,054	Examiner: Michael C. Wilson
Filed: January 8, 2001	Attorney Docket: 297/93/2
For: **Method of Producing an Undifferentiated Avian Cell Culture Using Avian Primordial Germ Cells**	

Appeal Brief to the Board of Patent Appeals
And Interferences under 37 C.F.R. § 41.37

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is an appeal pursuant to 35 U.S.C. § 134 from the Examiner's decision rejecting claims 44, 47, 48, 51-54, and 56-58 as set forth in the Final Official Action dated December 30, 2005.

I. Real Party in Interest – 37 C.F.R. § 41.37(c)(1)(i)

The real party in interest is North Carolina State University of Raleigh, North Carolina, United States of America.

II. Related Appeals and Interferences – 37 C.F.R. § 41.37(c)(1)(ii)

There are no appeals or interferences, known to Appellants or Appellants' legal representatives, which will directly affect or be directly affected by or have a bearing on

the decision by the Board of Patent Appeals and Interferences (hereinafter "the Board") in the pending appeal.

III. Status of the Claims – 37 C.F.R. § 41.37(c)(1)(iii)

Claims 44, 47, 48, 51-54, and 56-58 are pending in the subject application. Claims 44, 47, 48, 51-54, and 56-58 stand finally rejected as per the Final Official Action of December 30, 2005, and are the subject of this Appeal.

IV. Status of the Amendments – 37 C.F.R. § 41.37(c)(1)(iv)

A Final Official Action (hereinafter the "Final Official Action") was mailed by the United States Patent and Trademark Office (hereinafter "the Patent Office") on December 30, 2005. No After Final Amendments have been submitted by Appellants.

Additionally, a Declaration Pursuant to 37 C.F.R. § 1.132 by co-inventor Dr. James N. Petitte (hereinafter "the Petitte Declaration") was submitted on October 6, 2005. Although the Final Official Action did not address the Petitte Declaration, Appellants presume that the Petitte Declaration was entered into the record.

V. Summary of the Claimed Subject Matter – 37 C.F.R. § 41.37(c)(1)(v)

This summary is presented in compliance with the requirements of 37 C.F.R. § 41.37(c)(1)(v), mandating a "concise explanation of the subject matter defined in each of the independent claims involved in the appeal". Nothing stated within this summary is to be interpreted as changing the specific language of the claims, nor is the language of this summary intended to be construed so as to limit the scope of the claims in any way.

There is one independent claim pending in the application, claim 44. Claim 44 is directed to a sustained culture of undifferentiated chicken cells expressing an embryonic stem cell phenotype. The sustained culture comprises (a) a preconditioned feeder matrix; (b) conditioned media; (c) chicken primordial germ cells and chicken stromal cells; and (d) undifferentiated chicken cells expressing an embryonic stem cell phenotype. The chicken primordial germ cells and stromal cells are isolated together

from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system. The undifferentiated chicken cells (i) are derived from the chicken primordial germ cells isolated from the chicken embryo; (ii) are smaller than the chicken primordial germ cells; and (iii) form one or more colonies of tightly packed undifferentiated chicken cells expressing an embryonic stem cell phenotype.

Appellants are also separately arguing that certain of the dependent claims include elements that provide additional evidence of compliance of the same with the requirements of 35 U.S.C. § 112 and/or that provide an independent basis for distinguishing the claims from the cited references.

a) Claim 47: The preconditioned feeder matrix comprises cells that have been isolated from the gonad of a chicken embryo later than stage 14 according to the Hamburger & Hamilton staging system. Support in the specification can be found at page 11, lines 13-15.

b) Claim 48: The preconditioned feeder matrix comprises cells that have been isolated from the genital ridge of a chicken embryo later than state 14 according to the Hamburger & Hamilton staging system. Support in the specification can be found at page 11, lines 2-15.

c) Claim 51: The conditioned media is Buffalo Rat Liver (BRL) conditioned media. Support in the specification can be found at page 13, lines 10-15.

d) Claim 53: The embryonic stem cell phenotype is maintained for at least one month. Support in the specification can be found at page 14, lines 4-7.

e) Claim 54: The embryonic stem cell phenotype is maintained for at least two months. Support in the specification can be found at page 14, lines 4-7.

f) Claim 56: The preconditioned feeder matrix comprises mouse fibroblast cells. Support in the specification can be found at page 4, lines 15-17.

g) Claim 57: The mouse fibroblast cells comprise STO cells. Support in the specification can be found at page 4, lines 15-17.

h) Claim 58: The undifferentiated chicken cells maintain the embryonic stem cell phenotype when grown on the preconditioned fibroblast feeder matrix in the presence of the conditioned media for at least three days. Support in the specification can be found in Figure 4 and original claim 15.

VI. Grounds of Rejection to be Reviewed on Appeal – 37 C.F.R. § 41.37(c)(1)(vi)

The grounds of rejection for review are as follows:

A. Claims 44, 47, 48, 51-54, and 56-58 are subject to a new matter rejection under 35 U.S.C. § 112, first paragraph, upon the contention that the specification as filed did not contemplate maintaining the ES cell phenotype for one or two months.

B. Claims 44, 47, 48, 51-54, and 56-58 have been rejected under the enablement provision of 35 U.S.C. § 112, first paragraph.

C. Claims 44, 47, 48, 51-54, and 56-58 have been rejected under 35 U.S.C. § 112, second paragraph.

D. Claims 44, 47, 48, 52-54, and 58 have been rejected under 35 U.S.C. § 102(b) as being unpatentable over Chang (1995) 19 *Cell Biol Intl* 143-149.

E. Claims 44, 47, 48, 52-54, and 58 have been rejected under 35 U.S.C. § 102(b) as being unpatentable over Chang (1997) 21 *Cell Biol Intl* 495-499.

F. Claims 44, 47, 48, 51-54, and 56-58 have been rejected under 35 U.S.C. § 102(e) as being unpatentable over any of U.S. Patent Nos. 5,340,740; 5,656,479; or 5,830,510; all to Petitte *et al.*

G. Claims 44, 47, 48, 51-54, and 56-58 have been rejected under 35 U.S.C. § 103(a) over U.S. Patent No. 6,156,569 to Ponce de Leon in view of Chang (1995) 19 *Cell Biol Intl* 143-149.

H. Claims 44, 47, 48, 51-54, and 56-58 have been rejected under the judicially-created doctrine of obviousness-type double patenting over claims 1 and 8-10 of U.S. Patent No. 5,340,740 in view of the disclosure of U.S. Patent No. 5,340,740 and Chang (1995) 19 *Cell Biol Intl* 143-149.

I. Claims 44, 47, 48, 51-54, 56, and 57 have been rejected under the judicially-created doctrine of obviousness-type double patenting over claim 1 of U.S. Patent No. 5,656,479 or U.S. Patent No. 5,830,510 in view of Chang (1995) 19 *Cell Biol Intl* 143-149.

VII. Arguments – 37 C.F.R. § 41.37(c)(1)(vii)

A. New Matter Rejection under 35 U.S.C. § 112, First Paragraph (Claims 44, 47, 48, 51-54, and 56-58)

Claims 44, 47, 48, 51-54, and 56-58 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification as filed did not contemplate maintaining the ES cell phenotype for one or two months.

Initially, Appellants note that the Final Official Action includes assertions with respect to new matter only regarding claims 53 and 54. There are no allegations of new matter with respect to claims 44, 47, 48, 51, 52, and 56-58, nor do any of these claims depend directly or indirectly from either of claims 53 and 54. As a result, Appellants respectfully submit that the inclusion of claims 44, 47, 48, 51, 52, and 56-58 appears to be an oversight.

Accordingly, Appellants respectfully request that the Examiner withdraw the new matter rejection as to claims 44, 47, 48, 51, 52, and 56-58 in the Examiner's Answer. Alternatively, since the Examiner has provided no assertions to support a new matter rejection of claims 44, 47, 48, 51, 52, and 56-58, Appellants respectfully request that the Board reverse the instant rejection as to these claims for failure to establish a *prima facie* case of lack of compliance with 35 U.S.C. § 112, first paragraph, as it pertains to new matter.

With respect to claims 53 and 54, the Examiner asserts that the elements "wherein the embryonic stem cell phenotype is maintained for at least one month" and "wherein the embryonic stem cell phenotype is maintained for at least two months" constitute new matter.

Appellants respectfully disagree that the language at issue is new matter. The Board's attention is respectfully directed to page 13, line 21, through page 14, line 7 of the subject specification (particularly page 14, lines 4-7), which Appellants submit discloses a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype wherein the embryonic stem cell phenotype is maintained for at least one or two months. This section of the specification states:

> In a preferred embodiment, avian embryonic gonadal cells comprising primordial germ cells from a four to five day incubated avian embryo are seeded onto the preconditioned feeder matrix with conditioned media, and the avian cells give rise to nests or colonies of cells exhibiting an embryonic stem cell phenotype. Unlike the case with mammalian stem cells, it is currently preferred to have a preconditioned feeder matrix to

> facilitate the survival and development of avian PGCs into undifferentiated avian cells expressing an ESC phenotype. The avian embryo cells of the present invention can be cultured for at least one or two months as is typical for a primary cell culture, which is significantly greater than the usual two week life of primary cultures of cells from an unincubated avian embryo.

Specification at page 13, line 21, to page 14, line 7 (emphasis added).

The Examiner's position with regard to the instant rejection is exemplified by assertions presented in the Official Action dated April 7, 2004, in which the Examiner contended that the cited passage from the specification:

> merely states that PGCs are maintained for one or two months. The specification did not teach or suggest that cells having an ES cell phenotype were maintained in culture for one or two months and does not explicitly or implicitly suggest that the ES cell phenotype is maintained for one or two months as claimed. The specification did not teach or suggest the ES cell phenotype was maintained in culture for one to two months as claimed.

Official Action dated April 7, 2004 at page 4.

Appellants respectfully submit, however, that the Examiner's assertions do not establish a *prima facie* case in support of the instant rejection. According to M.P.E.P. § 2163.04:

> A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See, e.g., *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976).

M.P.E.P. § 2163.04 (emphasis added).

Additionally, M.P.E.P. § 2164 further indicates that there is a strong presumption that the written description is adequate. Thus, Appellants respectfully submit that the Examiner has the burden of demonstrating by a preponderance of the evidence that

one of ordinary skill in the art would not understand the specification as filed to disclose that the embryonic stem cell phenotype could be maintained for one or two months.

Appellants respectfully submit that the Examiner has provided no such preponderance of the evidence. Rather, Appellants respectfully submit that in support of the instant rejection, the Examiner asserts that "the absence of maintaining the stem cell phenotype for at least one or two months as claimed is adequate 'evidence or technical reasoning' that Appellants did not disclose the subject matter at the time of filing" (Final Official Action at pages 2-3, bridging paragraph). However, Appellants respectfully submit that this "reasoning" amounts to no more than a circular argument. Appellants respectfully submit that this assertion does not rise to the level of a preponderance of evidence as required by M.P.E.P. § 2163.04, and thus the instant rejection must be reversed.

Appellants further respectfully submit that support for new and/or amended claims need not be found explicitly in the specification as filed. Rather, support can also be present implicitly or inherently in the disclosure (*see* M.P.E.P. § 2163). Appellants respectfully submit that one of ordinary skill in the art would understand after consideration of the specification as a whole that the specification discloses maintaining the embryonic stem cell phenotype for at least one or two months.

To elaborate, the instant specification at page 14, lines 4-7, discloses that the avian embryo cells of the present invention can be cultured for at least one or two months. The specification further discloses at page 1, lines 5-8, that "the present invention relates to undifferentiated avian cells expressing an embryonic stem cell phenotype in general, and particularly relates to avian primordial germ cells and undifferentiated avian cells expressing an embryonic stem cell phenotype" (emphasis added). Thus, the specification discloses that the avian embryo cells of the present invention can be cultured for one or two months and further that the present invention relates to undifferentiated avian cells expressing an embryonic stem cell phenotype. Putting these two exemplary sections together clearly indicates that the "present invention" relates to "undifferentiated avian cells expressing an embryonic stem cell phenotype" that can be "cultured for at least one or two months".

> Furthermore, page 3, lines 8-16, of the instant specification discloses that:
>
> Undifferentiated avian cells expressing an ESC phenotype are useful, among other things, as a tool for the study of embryological development (i.e., by labeling the cells with a marker gene and observing their distribution after injection in vivo) and the production of transgenic poultry. They are useful in allowing the application of homologous recombination to the production of transgenic poultry. In view of such uses, the development of additional methods for obtaining undifferentiated avian cells expressing an ESC phenotype represents a continuing need in the art.

Specification at page 3, lines 6-16 (emphasis added). Thus, Appellants respectfully submit that one of ordinary skill in the art would understand after consideration of the instant specification that the presently disclosed subject matter is directed to undifferentiated avian cells that can be used, for example, for studies of developmental biology (*e.g.*, they can be labeled with a marker gene in order to observe their distribution after injection *in vivo*) and/or for the production of transgenic poultry. *See e.g.*, the Petitte Declaration, Point 10). Additionally, they can be used for homologous recombination strategies in poultry.

In each case, Appellants respectfully submit that the establishment and maintenance of the undifferentiated state are aspects of the presently disclosed subject matter in that when the cells are undifferentiated they would be expected to take part in the normal development of various different cell and tissue types in a chimeric animal. One of ordinary skill in the art would understand that if one wishes, for example, to perform cell fate experiments with labeled cells and/or to perform knockout experiments, it is desirable that the cells be undifferentiated so that they are capable of developing normally into different cell types in recipient animals. Differentiated cells by definition lack this ability, and thus are of little or no use in cell fate experiments and homologous recombination experiments.

Therefore, Appellants respectfully submit that when one of ordinary skill in the art considers the specification as a whole, he or she would understand that it is a goal of the presently disclosed subject matter to produce such undifferentiated cells. Appellants further respectfully submit that upon a review of the present disclosure, one of ordinary skill in the art would also understand that once undifferentiated cells are

produced, it is desirable that the cells be maintained in an undifferentiated state, and that this can be ensured by control of the culturing conditions. Given the desirability of maintaining the undifferentiated state, Appellants respectfully submit that one of ordinary skill in this art would also know that should the cells begin to differentiate, they would no longer be suitable for use in these types of experiments, and thus the cultures would be discarded.

Turning now to the Examiner's assertions in support of the present rejection, it appears that the Examiner contends that the specification as filed discloses that the embryo cells themselves (*i.e.*, the primordial germ cells (PGCs)) could be maintained in culture for 1-2 months, but that there is no disclosure that the ES cell (ESC) phenotype is also maintained for 1-2 months. Appellants respectfully disagree. Given that the point of establishing the sustained cultures is not to generate long term cultures of PGCs but to establish derivatives of PGCs in culture that are undifferentiated and express an ESC phenotype, Appellants respectfully submit that the only reasonable interpretation for the cited passage is that PGCs are cultured under conditions sufficient to facilitate the survival and development of undifferentiated avian cells expressing an ESC phenotype from said PGCs.

Furthermore, the specification discloses that the PGC-derived undifferentiated cells are present in the sustained culture after about three or five days under the disclosed culture conditions (*see* the descriptions for Figures 4A-4F on page 7 of the specification; *see also* the Examples, particularly Example 3 on page 21, lines 6-11). Once colonies are established, one of ordinary skill in the art would understand that the continued culture of the cells would be for the purpose of confirming that they can be maintained in an undifferentiated state so that they would be good candidates for the uses described hereinabove. If, as the Examiner appears to contend, the cells did not generate colonies of undifferentiated cells within the first 3-5 days or if they did but subsequently lost their status as undifferentiated cells, one of ordinary skill in the art would understand that the cultures would have been abandoned, not cultured until one or two months had passed.

Stated another way, Appellants respectfully submit that it would have been self-evident to one of ordinary skill in the art upon a review of the present disclosure that if the desired outcome (establishment of colonies of undifferentiated cells) did not occur within the first 3-5 days, or alternatively if it did occur but that the undifferentiated state was subsequently lost, an additional 25-60 days of culturing would have been completely futile for either capturing or re-capturing the undifferentiated state, and thus there would have been no purpose in continuing to culture the cells. As such, Appellants respectfully submit that one of ordinary skill in the art would understand after consideration of the specification as a whole that the continuation of the cultures for one or two months relates to the sustained culture of the undifferentiated cells. Thus, the avian embryo cells that can be cultured for at least one or two months are the undifferentiated avian cells expressing an ESC phenotype.

Summarily, Appellants respectfully submit that the cells present in the sustained cultures of claims 44 and dependents thereof can be maintained in an undifferentiated state for at least one or two months. Appellants respectfully submit that the overall teaching of the specification involves the establishment of such cells, and that the cells' status as being undifferentiated is relevant to envisioned uses. If the cells lost their status as undifferentiated (*e.g.*, if the cells in the culture began to differentiate during the one or two month culture) and/or if colonies of undifferentiated cells were not established to begin with (*i.e.*, the PGCs remained as PGCs *per se*), Appellants respectfully submit that there would have been no disclosure in the specification concerning the cultures at the one to two month time points because the cultures would have been discarded prior to these time points.

Therefore, Appellants respectfully submit that one of ordinary skill in the art would understand this to be the case based on the specification as a whole. As such, Appellants respectfully submit that the Examiner has not presented a preponderance of evidence that claims 53 and 54 contain new matter as is required under M.P.E.P. § 2163.04.

Accordingly, Appellants respectfully request that the Board reverse the instant rejection of claims 53 and 54 under 35 U.S.C. § 112, first paragraph.

B. Enablement Rejection under 35 U.S.C. § 112, First Paragraph (Claims 44, 47, 48, 51-54, and 56-58)

The Examiner has rejected claims 44, 47, 48, 51-54, and 56-58 under the enablement provision of 35 U.S.C. § 112, first paragraph. According to the Final Official Action, the specification as filed does not enable maintaining chicken ES cells or any other chicken ES cells for at least one or two months as claimed.

Initially, Appellants respectfully submit that as with the rejection discussed immediately hereinabove, it does not appear that the instant rejection is intended to be, or in fact can properly be, applied to claims 44, 47, 48, 51, 52, and 56-58. The only basis for the instant rejection relates to the elements concerning maintaining the ES cell phenotype for one or two months, which appear only in claims 53 and 54.

Accordingly, it appears that this rejection should in fact be applied only to claims 53 and 54. As such, Appellants respectfully request that the Examiner withdraw the rejection as to these claims in his Answer. Alternatively, Appellants respectfully submit that the Examiner has presented no support for the application of the instant rejection to claims 44, 47, 48, 51, 52, 54, or 56-58, and respectfully request that the Board reverse the rejection as to these claims.

Continuing with the instant rejection as applied to claims 53 and 54, on page 4 of the Final Official Action, the Examiner asserts that Simkiss (1990, 16 *4th World Congr Genetic Appl Livestock Prod* 111-114; hereinafter "Simkiss") and Petitte (1990, 108 *Development* 185-195; hereinafter "Petitte 1990") teach chicken PGCs capable of producing somatic and germline chimeras. The Examiner further asserted that Ponce de Leon (1997, 21 *Revista Brasiliera de Reproducao Animal* 96-101; hereinafter "Ponce de Leon 1997") taught that LIF, bFGF, IGF, and SCF are required for long term culture of chicken PGCs. The Examiner asserted that the PGCs of Ponce de Leon 1997 "are ES cells because they provide germ and somatic cell chimeras upon being introduced into recipient embryos" (Final Official Action at page 4). The Examiner thus asserted that the art did not teach how to culture chicken PGCs having an ES cell phenotype for one or two months.

Appellants respectfully submit that the references cited by the Examiner and the assertions presented in the Final Official Action do not support the instant rejection, and in fact are irrelevant to the enablement of the claims. For example, Appellants respectfully submit that Simkiss, Petitte 1990, and Ponce de Leon 1997 disclose the culture of PGCs *per se*, not the culture of an undifferentiated derivative of a PGC that is the subject of the instant application. Accordingly, Appellants respectfully submit that whatever culture conditions might have been disclosed in the cited references for the long term culture of PGCs, these conditions do not inform the skilled artisan concerning how to culture undifferentiated cells derived from PGCs for one or two months because the cells of the instant claims are not PGCs.

The Examiner's misunderstanding of the subject matter of the claims is further evidenced by the assertion that "the art did not teach how to culture chicken PGCs having an ES cell phenotype for one or two months". Appellants respectfully submit that chicken PGCs do not have an ES cell phenotype as that phrase is employed in the instant claims. This assertion appears to be based on the Examiner's contention that Simkiss, Petitte 1990, and Ponce de Leon 1997 disclose chicken PGCs that are capable of producing somatic and germline chimeras. Appellants respectfully submit that Simkiss, Petitte 1990, and Ponce de Leon 1997 do not disclose PGCs capable of producing somatic chimeras at all, and thus the Examiner's contention represents an inaccurate reading of these references.

Turning first to the disclosure of Simkiss, Appellants respectfully submit that this reference teaches only that retroviral sequences present in transferred PGCs were incorporated into the genome of the gonad (*see* Simkiss at page 111, Summary). There is no disclosure that unequivocally supports the contention that the transferred PGCs colonized any somatic tissue in the embryo. It appears, however, that the Examiner is asserting that the disclosure relating to the detection of retroviral sequences in the anterior half of the 5 day embryo is indicative of somatic contribution of the transferred PGCs in the recipient since the gonad of the 5 day embryo is in the posterior half (*see* Simkiss at page 112, third paragraph).

However, Appellants respectfully submit that the apparent conclusion that this represents evidence of somatic chimerism is misplaced. Appellants respectfully submit that it is known in the art that PGCs transferred to recipient embryos do not result in somatic chimerism, but rather home to the gonads where they best they produce germline chimeras (*see e.g.*, the Petitte Declaration, Point 11). In view of this understanding, Appellants respectfully submit that the appearance of a positive signal in the anterior half of the 5 day embryo in Simkiss is not evidence of somatic chimerism but is likely a consequence of the nature of the transfer and of the screening method employed.

To elaborate, Simkiss discloses that PGCs were transferred to the circulatory system of recipient embryos (*see* Simkiss at page 111, Summary). From here, the PGCs would be expected to migrate through the circulatory system to the embryonic gonad and/or genital ridge (*see* Petitte Declaration, Point 11; *see also* the Example beginning at column 7 of U.S. Patent No. 6,156,569 to Ponce de Leon *et al.*, including particularly column 8, lines 7-9, and column 8, line 54 through column 9, line 5). The polymerase chain reaction (PCR) was then used to screen total DNA isolated from the anterior and posterior halves of the embryo for the presence of a retroviral-encoded sequence (*i.e.*, a *lacZ* gene encoded by a recombinant retrovirus). The posterior half was positive for *lacZ*, which was expected since the presumptive gonad was present in the posterior half (*see* Simkiss at page 112, third paragraph).

The presence of a positive signal in the anterior half of the embryo is unexpected. The Examiner appears to assert that this is indicative of somatic chimerism because there are no gonadal tissues in the anterior half of these embryos, but Appellants respectfully submit that this conclusion is not warranted based on the evidence presented in Simkiss in view of the understanding of one of ordinary skill in the art. Rather, Appellants respectfully submit that it is likely that the positive signal in the anterior half of the embryo results from the presence of PGCs that are still in the circulation of the embryos at this stage, and thus are still present in the anterior regions of the circulatory system at the time the embryos were recovered. Given the sensitivity of PCR, Appellants respectfully submit that even a single PGC present in the circulatory

system in the anterior half of these embryos would be expected to generate a positive signal in the anterior half of the embryos.

Furthermore, Appellants respectfully submit that the migration of PGCs to the gonad after transfer would not be expected to be 100% efficient, thereby resulting in at least some of the transferred cells remaining in the circulation. In fact, Simkiss discloses on page 113 in the Discussion that "when infected PGCs are transferred to the embryo they become progressively localized in the germinal crescent.

Summarily, Appellants respectfully submit that the Examiner's assertion that Simkiss disclosed somatic chimeras after PGC transfer is not supported by the weight of the evidence. The PCR reaction performed on the anterior halves does not indicate that the transferred PGCs contributed to any somatic tissue present in the anterior half of the embryos. Appellants respectfully submit that in the absence of any evidence in Simkiss as to where the specific cells that contained the *lacZ* sequences were located and since the Examiner's assertions are contrary to the understanding of the skilled artisan with respect to how PGCs behave after transfer to recipient embryos, the Examiner's conclusions with respect to somatic chimerism are unwarranted. Accordingly, Appellants respectfully submit that contrary to the Examiner's assertions in support of the instant rejection, Simkiss does not disclose that PGCs are capable of producing somatic chimeras.

Turning now to Petitte 1990, Appellants respectfully submit that this reference does not support the instant rejection because there is no disclosure in Petitte 1990 that the cells that were responsible for the somatic chimerism were PGCs. Rather, Appellants respectfully submit that Petitte 1990 discloses the transfer of cells isolated from whole blastoderms from stage X embryos into recipient embryos (*see* Petitte 1990, pages 185-186, bridging paragraph). The actual cell type that gave rise to the somatic chimerism seen in the recipient embryos is not identified, and given the understanding by one of ordinary skill in the art that PGCs *per se* migrate to the embryonic gonad and localize therein, Appellants respectfully submit that the Examiner's apparent assertion that the cells that generated the somatic chimeras in Petitte 1990 were in fact PGCs finds no support whatsoever in the reference. Therefore, Appellants respectfully submit

that Petitte 1990 does not support the Examiner's assertion that PGCs can give rise to somatic chimeras after transfer into recipient embryos.

With respect to Ponce de Leon 1997, Appellants respectfully submit that this reference also fails to teach that PGCs are capable of giving rise to somatic chimeras. In support of this assertion, the Examiner cited page 100, lines 1-7, of the Results and Discussion section of Ponce de Leon 1997. This passage discloses that "Chimeric chickens generated from fresh and cryopreserved PGCs. Twenty-five (74%) out of 34 putative chimeric chicken, produced with fresh PGCs transfers, prove to be true chimeric animals after progeny testing" (Ponce de Leon 1997 at page 100, final paragraph; emphasis added).

Appellants respectfully submit that given that this passage discloses that chimerism was proven by progeny testing, it is clear that the chimerism being tested was germline chimerism and not somatic chimerism. Appellants respectfully submit that progeny testing cannot generate any information concerning somatic chimerism since only germline contribution can be tested in progeny.

Stated another way, Appellants respectfully submit that one of ordinary skill in the art would understand that what progeny testing is intended to reveal is whether or not a putative chimera is a germline chimera. Therefore, and contrary to the Examiner's assertion, Appellants respectfully submit that Ponce de Leon 1997 does not disclose any somatic chimeras, and thus the Examiner's assertion that Ponce de Leon 1997 discloses chicken PGCs capable of producing somatic and germline chimeras reflects a inaccurate understanding of Ponce de Leon 1997.

Therefore, Appellants respectfully submit that none of the references relied on by the Examiner discloses any ability of PGCs to produce somatic chimeras. Accordingly, the Examiner's assertion that "the PGCs of Ponce de Leon are ES cells because they provide germ and somatic cell chimeras upon being introduced into recipient embryos" on page 4 of the Final Official Action is believed to be inaccurate and unsupported by the reference. Therefore, Appellants respectfully submit that each of Simkiss, Petitte 1990, and Ponce de Leon 1997 fails to support the instant rejection.

Turning now to the second assertion upon which the instant rejection is based, Appellants respectfully submit that the Examiner's assertion that "the specification does not teach the amounts of essential growth factors required to culture chicken ES cells in the presence of feeder cells for one or two months" also fails to support the instant rejection. This assertion is based on the contention that Ponce de Leon 1997 teaches that LIF, bFGF, IGF, and SCF are essential to culture ES cells long term.

Appellants respectfully disagree. Appellants respectfully submit that Ponce de Leon 1997 discloses long term culture of PGCs, and thus does not disclose long term culture of ES cells. Since PGCs are not equivalent to either ES cells or to the undifferentiated chicken cells expressing an embryonic stem cell phenotype of claim 44, Appellants respectfully submit that culture conditions that might or might not be required for long term culture of PGCs as disclosed in Ponce de Leon 1997 are irrelevant to the examination of the subject matter of the instant claims.

To elaborate, the instant rejection appears to be based on the assertion that the PGCs of Ponce de Leon 1997 are ES cells, and thus have an ES cell phenotype. Appellants respectfully submit that they have rebutted this contention since contrary to the Examiner's assertion, Ponce de Leon 1997 does not teach an ability of PGCs to contribute to somatic lineages in a chimera. Thus, PGCs do not have this alleged "ES cell phenotype".

Additionally, claim 44 recites that the undifferentiated chicken cells (i) are derived from chicken primordial germ cells isolated from the chicken embryo; (ii) are smaller than chicken primordial germ cells; and (iii) form one or more colonies of tightly packed undifferentiated chicken cells expressing an embryonic stem cell phenotype. As such, Appellants respectfully submit that in the language of claim 44, Appellants have distinguished the claimed undifferentiated chicken cells structurally from PGCs. Thus, Appellants respectfully submit that references that disclose culture conditions for chicken PGCs are of little or no instructive value with respect to the instantly claimed cultures since one of ordinary skill in the art would understand that PGCs and ES cell are different cells types and are cultured under different conditions.

Next, the Examiner asserts that the citation on page 14, lines 4-5, of the instant specification does not describe how to maintain the ES cell phenotype for one to two months as claimed. This assertion appears to be based on an assertion that "the specification does not teach the amounts of essential growth factors required to culture chicken ES cells in the presence of feeder cells for one or two months" (Final Official Action at page 4), which is in turn itself based on the disclosure in Ponce de Leon 1997 that certain growth factors are required for the long term culture of PGCs. However, the undifferentiated chicken cells of claim 44 are not PGCs, and thus Appellants respectfully submit that whatever culture conditions might be required for long term PGC culture, such as the addition of LIF, bFGF, IGF, SCF, and/or any other growth factors, this disclosure does not imply that these same growth factors are required for long term culture of the instantly claimed cells, which are not PGCs.

Appellants respectfully submit that the specification as filed discloses culture conditions that have been shown to be sufficient for the generation and culture of the claimed cells. These conditions are disclosed in the Examples. The specification further discloses that the cells can be cultured for at least one or two months. As discussed hereinabove, the specification would be understood by one of ordinary skill in the art to disclose that the undifferentiated state is maintained for at least this culture period. Appellants respectfully submit that that one of ordinary skill in the art would understand after review of the instant specification that culturing isolated PGCs from a later than stage 14 embryo under the culture conditions disclosed will generate the undifferentiated chicken cells of claims 44 and dependents thereof.

Thus, Appellants respectfully submit that the Examiner has failed to meet his burden under M.P.E.P. § 2164.04, which states in relevant part that:

> A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

(emphasis added). M.P.E.P. § 2164.04 further states that:

> As stated by the court [in *In re Marzocchi* (439 F.2d 220, 224, 169 USPQ 367 (CCPA 1971)], "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." 439 F.2d at 224, 169 USPQ at 370.

M.P.E.P. § 2164.04 (emphasis added). Appellants respectfully submit that the Examiner has provided no such "acceptable evidence or reasoning" to doubt the objective truth of the statements, since reliance on references that disclose culture conditions for a different cell type (a PGC) cannot be deemed to be sufficient to overcome the evidence provided in the working examples of the instant application.

Summarily, Appellants respectfully submit that the instant rejection is based on several conclusory and unsubstantiated assertions concerning the state of the prior art and its applicability to the presently disclosed subject matter. Because these assertions do not overcome the presumption that the disclosure is enabling as per M.P.E.P. § 2164.04 given the culture conditions disclosed in the instant specification, Appellants respectfully request that the Board reverse the instant rejection.

C. Rejection under 35 U.S.C. § 112, Second Paragraph (Claims 44, 47, 48, 51-54, and 56-58)

Claims 44, 47, 48, 51-54, and 56-58 have also been rejected under 35 U.S.C. § 112, second paragraph, upon the contention that several phrases appearing in the claims are indefinite. More particularly, the Examiner has rejected the claims based on the following assertions:

1. the cells encompassed by the phrase "undifferentiated chicken cells expressing an embryonic stem cells phenotype" are unclear; and
2. it is unclear how PGCs isolated from an embryo later than stage 14 are distinguished from PGCs isolated from a stage X or a stage 14 embryo.

Turning first to the nature of the phrase "undifferentiated chicken cells expressing an embryonic stem cells phenotype", Appellants respectfully submit that the Examiner

appears to be examining this phrase in isolation and outside of the context of the specification and claims, particularly when viewed from the perspective of one of ordinary skill in the art after consideration of the specification as a whole. Appellants respectfully submit that this failure to consider the phrase at issue from the skilled artisan's perspective after consideration of the specification as a whole results in the instant rejection being fatally flawed.

To elaborate, Appellants respectfully submit that the phrase at issue appears in claim 44, which recites a sustained culture of undifferentiated chicken cells expressing an embryonic stem cell phenotype, the sustained culture comprising:

(a) a preconditioned feeder matrix;

(b) conditioned media;

(c) chicken primordial germ cells and chicken stromal cells, wherein the chicken primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system; and

(d) undifferentiated chicken cells expressing an embryonic stem cell phenotype,

wherein the undifferentiated chicken cells:

(i) are derived from the chicken primordial germ cells isolated from the chicken embryo;

(ii) are smaller than the chicken primordial germ cells; and

(iii) form one or more colonies of tightly packed undifferentiated chicken cells expressing an embryonic stem cell phenotype.

Thus, Appellants respectfully submit that the "undifferentiated chicken cells expressing an embryonic stem cell phenotype" are derived from the chicken primordial germ cells (PGCs) isolated from a chicken embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system and are smaller than the chicken primordial germ cells so isolated. More particularly, Appellants respectfully submit that the cells are derived from PGCs by culturing PGCs *in vitro*. The derivatives are not PGCs

themselves, as they are smaller than PGCs. Additionally, the undifferentiated chicken cells form one or more colonies of tightly packed cells, which is also unlike the behavior of PGCs in culture.

Therefore, one of ordinary skill in the art would understand from the disclosure that the undifferentiated chicken cells expressing an embryonic stem cell phenotype are morphologically distinguishable from PGCs. This morphological distinction is intended by Appellants to be encompassed by the phrase "expressing an ES cell phenotype", which the specification as filed defines as referring to "undifferentiated avian cells having a large nucleus, a prominent nucleolus, and little cytoplasm" (*see* page 9, lines 4-5). The specification further discloses on page 9, lines 19-22, that the phrase "undifferentiated avian cell expressing an embryonic stem cell phenotype encompasses cells derived from avian primordial germ cells and is therefore used to describe the cells cultured in accordance with the process of the present invention".

The Examiner concedes that the specification as filed defines this phrase, but asserts that "such a description is ambiguous because it cannot be determined what Appellants consider 'large', 'prominent' or 'little'" (Final Official Action age page 7). Appellants have maintained that this description would be understood to those of skill in the art as being a morphological characterization of ES cells and ES-like cells derived from PGCs.

In fact, the Examiner appeared to concede on page 13 of the Official Action dated April 6, 2005 that one of ordinary skill in the art would indeed understand the metes and bounds of this phrase as it relates to ES cells. Further, the Board's attention is directed to page 9, lines 6-22 of the instant specification, which disclose the following:

> It has been reported that mouse PGCs maintained on STO feeder cell monolayers in the presence of LIF and bFGF resulted in cells resembling embryonic stem cells. See Resnick et al. *Nature* 359:550-551 (1992); Matsui et al., *Cell* 70:841-843 (1992). Resnick et al. *Nature* 359:550-551 (1992) suggested that such cells be designated "embryonic germ" (EG) for convenience cells to imply that they originated from PGCs *in vitro*. However, it is recognized among those of ordinary skill in the art that embryonic germ cells and embryonic stem cells are phenotypically the same in that they appear to be the same upon microscopic inspection (despite reported differences in methylation of some genes), display the same immunological markers, and are functionally the same in that both

> have been shown to differentiate extensively in culture and to contribute to chimeras when injected into host blastocysts, thus demonstrating their pluripotent and totipotent nature. Accordingly, the phrase "undifferentiated avian cell expressing an embryonic stem cell phenotype" encompasses cells derived from avian primordial germ cells and is therefore used to describe the cells cultured in accordance with the process of the present invention.

Specification at page 9, lines 6-22 (emphasis added).

As such, Appellants respectfully submit that the Examiner's apparent concession that one of ordinary skill in the art would understand that the phrase "a large nucleus, a prominent nucleolus, and little cytoplasm" is a morphological description of ES cells in combination with the specification's disclosure that one of ordinary skill in the art would understand embryonic germ cells (*i.e.*, ES-like cells derived from PGCs) and embryonic stem cells to be phenotypically the same undermines the assertion upon which the instant rejection is based; namely that "it cannot be determined what Appellants consider 'large', 'prominent' or 'little'." Since it is axiomatic that the language of the claims must be viewed from the perspective of one of ordinary skill in the art in view of the specification as a whole, Appellants respectfully submit that the Examiner has not presented a *prima facie* case that claim 44 fails to comply with 35 U.S.C. § 112, second paragraph.

Turning now to the additional assertions presented by the Examiner in the Final Official Action with respect to this rejection, Appellants respectfully submit that the contention that "the description is also ambiguous because a phenotype cannot be defined as cells" is misleading. This assertion appears to relate to the definition presented on page 9, lines 4-5 of the specification as filed, which states that "The phrase 'embryonic stem cell phenotype' refers to undifferentiated avian cells having a large nucleus, a prominent nucleolus, and little cytoplasm". Appellants respectfully submit that one of ordinary skill in the relevant art, who would thus be familiar with the culture of cells such as ES cells and PGC-derived undifferentiated cells, would understand that this description relates to a morphological description (which is undeniably a "phenotype") of such cells. Appellants therefore respectfully submit that one of ordinary skill in the art would understand the nature of the "phenotype" and thus

would understand the metes and bounds of the subject matter of claim 44. Thus, Appellants respectfully submit that the Examiner's assertion does not support the instant rejection of claim 444.

Accordingly, Appellants respectfully submit that one of ordinary skill in the art would understand the metes and bounds of the phrase at issue when viewed in the context of the claims and the specification as a whole. Appellants further respectfully submit that this is all that is required under the jurisprudence of the United States Court of Appeals for the Federal Circuit as set forth in *Phillips v. AWH Corp.*, 415 F.3d 1303, 75 U.S.P.Q.2d 1321 (Fed.Cir. 2005), which held:

> the person of ordinary skill in the art is deemed to read the claim term not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the specification. This court explained that point well in *Multiform Desiccants, Inc. v. Medzam, Ltd.*, 133 F.3d 1473, 1477 (Fed.Cir. 1998): It is the person of ordinary skill in the field of the invention through whose eyes the claims are construed.

415 F.3d at 1313. Therefore Appellants respectfully submit that the first assertion presented in support of the instant rejection is believed to be improper.

Turning now to the second assertion presented in support of the instant rejection, the Examiner contends that it is unclear how PGCs isolated from an embryo later than stage 14 are distinguished from PGCs isolated from a stage X or a stage 14 embryo. According to the Examiner, PGCs isolated from stage X, 14, and after stage 14 embryos have the same function as supported by Ponce de Leon 1997, Petitte 1990, and/or Naito (of record). These references are asserted to disclose that each of these PGC preparations were capable of germline transmission upon being transplanted into a recipient embryo, and as such chicken PGCs isolated before and after stage 14 are functionally equivalent.

Initially, Appellants respectfully submit that the Naito reference cited in the Final Official Action on page 10 has not in fact been made of record. Appellants have been unable to find any reference to this publication, and thus have not been able to review the publication in detail.

Additionally, Appellants respectfully submit that there are two main art-recognized staging systems, the Eyal-Giladi and Kochav (EG&K; from Eyal-Giladi and Kochav, 1976, Dev. Biol. 49(2):321-37) staging system and the Hamburger and Hamilton (H&H; from Hamburger & Hamilton, 1951, J Morphol 88:49-92) staging system. The former uses Roman numerals, and the latter Arabic numerals. The EG&K system relates to those stages prior to formation of the primitive streak, whereas stage 1 of the H&H system begins after the egg is laid and incubation begins. At that time the embryonic a flat disk of about 50,000 cells. Thus, Appellants respectfully submit that stage X, for example, occurs about 40 hours earlier than stage 14 in chickens.

Continuing with the instant rejection, Appellants respectfully submit that even assuming *arguendo* that PGCs from stage 13 to 14 as disclosed in Ponce de Leon 1997 or from stage 13-15 as apparently disclosed in the Naito reference were capable of germline transmission after transfer into a recipient, the presently disclosed subject matter does not relate to PGCs *per se*. Rather, it is clear from the disclosure and from the express language of the claims that the presently disclosed subject matter relates to derivatives of chicken PGCs that are smaller than chicken PGCs and that form one or more colonies of tightly packed undifferentiated chicken cells expressing an embryonic stem cell phenotype. Thus, the disclosures of the behaviors of PGCs *per se* in Ponce de Leon 1997 and/or apparently in the Naito reference are not believed to be relevant to whether or not there are structural and/or functional differences between the instantly claimed undifferentiated PGC derivatives and PGCs themselves.

Furthermore, with respect to Petitte 1990, Appellants respectfully submit that contrary to the Examiner's assertion, this reference does not teach the isolation of stage X PGCs. Rather, it teaches the isolation of entire stage X blastoderms.

Irrespective of the above, the same comments presented hereinabove with respect to Ponce de Leon 1997 and the Naito reference are equally applicable to Petitte 1990. Particularly, the behavior of PGCs and/or blastoderm cells do not inform one of ordinary skill in the art about the structural and/or functional characteristics of the PGC derivatives claimed in the instant application.

It would appear, therefore, that the Examiner is equating PGCs with the undifferentiated cells of the claimed sustained cultures. Appellants respectfully submit, however, that this is clearly in error since the claims themselves explicitly recite certain structural differences between PGCs and undifferentiated cells of the presently claimed sustained cultures. Particularly, Appellants respectfully submit that claim 44 recites *inter alia* that the undifferentiated chicken cells present in the sustained cultures (i) are derived from chicken primordial germ cells; (ii) are smaller than the chicken primordial germ cells; and (iii) form one or more colonies of tightly packed undifferentiated chicken cells expressing an embryonic stem cell phenotype, which is unlike chicken primordial germ cells.

Summarily, Appellants respectfully submit that none of the assertions presented by the Examiner support the rejection of Claims 44, 47, 48, 51-54, and 56-58 under 35 U.S.C. § 112, second paragraph. Thus, Appellants respectfully submit that the instant rejection is improper for the reasons set forth hereinabove, and respectfully request that it be reversed.

D. Rejection under 35 U.S.C. § 102(b) over Chang 1995 (Claims 44, 47, 48, 52-54, and 58)

Claims 44, 47, 48, 52-54, and 58 have been rejected under 35 U.S.C. § 102(b) as being unpatentable over Chang 1995. This rejection was maintained from the previous Official Action dated April 6, 2005 for the reasons presented therein. According to this Official Action, Chang 1995 is asserted to teach making feeder cells by isolating cells from the genital ridge of day 5 embryos and culturing the cells for 4 days. The Examiner further asserted that the feeder cells are "preconditioned" because they are in culture for 4 days prior to the addition of day 2 PGCs, that the feeder cell media is "conditioned" because it contains biologically active components obtained from the previous 4 days in culture prior to adding day 2 PGCs, and that the cells isolated from the genital ridge comprised stromal cells and PGCs as claimed.

Even assuming *arguendo* that each of these assertions is accurate, Appellants respectfully submit that Chang 1995 does not support an anticipation rejection of the

claims because Chang 1995 does not disclose each and every element of the claims. Specifically, Chang 1995 does not disclose the production of a sustained culture comprising undifferentiated chicken cells, wherein the undifferentiated chicken cells (i) are derived from chicken PGCs isolated from the genital ridge or gonad of a later than stage 14 embryo, (ii) are smaller than the chicken PGCs so isolated; and (iii) form one or more colonies of tightly packed undifferentiated chicken cells that express an embryonic stem cell phenotype. Rather, Appellants respectfully submit that Chang 1995 discloses cultures of PGCs *per se*, the cells of which are then transferred into chicken embryos where they colonize only the gonad of the recipient chicken.

Stated another way, Chang 1995 discloses removing PGCs, culturing them as PGCs in the short term, and returning them to a recipient where they colonize only the gonad. Since PGCs normally colonize the gonad, Chang 1995 does not disclose a change in either the phenotype of or the behavior of the isolated cells (*i.e.*, the PGCs).

In contrast, claim 44 of the instant application recites a sustained culture comprising *inter alia* one or more colonies of tightly packed undifferentiated cells that are derived from PGCs, each individual cell of which is smaller than a PGC. Thus, it is clear from the language of claim 44 that the sustained cultures comprise a cell type that is derived from PGCs but that are morphologically and phenotypically distinct from PGCs. Appellants respectfully submit that this is in contrast to the cited Chang 1995 reference, which teaches a short-term culture of PGCs, not a culture that includes undifferentiated derivatives of PGCs that are smaller than PGCs and form tightly packed colonies.

Since Chang 1995 does not disclose each and every element of claim 44, Appellants respectfully submit that Chang 1995 does not anticipate claim 44. Claims 47, 48, 52-54, and 58 all depend directly or indirectly from claim 44, and thus include all the elements of distinguished claim 44. Accordingly, Appellants respectfully request the reversal of the rejection of claims 44, 47, 48, 52-54, and 58 in view of Chang 1995.

Additionally, Appellants respectfully submit that Chang 1995 does not disclose the elements of dependent claims 53, 54, or 58. Particularly, Appellants respectfully submit that since Chang 1995 does not disclose maintaining the undifferentiated state

for at least one (claim 53) or two (claim 54) months or maintaining the embryonic stem cell phenotype when the cells are grown on the preconditioned fibroblast feeder matrix in the presence of the conditioned media for at least three days (claim 58).

Therefore, while claims 53, 54, and 58 are believed to be distinguished over Chang 1995 by virtue of their dependence from distinguished claim 44, these claims are also believed to be distinguished from this reference as a result of the additional elements recited in the claims. As such, Appellants respectfully submit that claims 53, 54, and 58 are all independently distinguishable over Chang 1995.

E. Rejection under 35 U.S.C. § 102(b) over Chang 1997 (Claims 44, 47, 48, 52-54, and 58)

Claims 44, 47, 48, 52-54, and 58 have been rejected under 35 U.S.C. § 102(b) upon the contention that the claims are anticipated by Chang 1997. According to the Examiner, Chang 1997 teaches isolating germinal ridge stromal cells from stage 27-28 embryos, which were then cultured for 5 days in media containing IGF, FGF, and LIF with germinal ridge stromal feeder cells isolated from day 5 embryos to obtain gPGCs.

Appellants respectfully submit that Chang 1997 does not support the instant anticipation rejection of the claims for precisely the same reasons that are outlined immediately hereinabove with respect to Chang 1995. In particular, Chang 1997 does not teach a sustained culture comprising *inter alia* PGC derivatives that comprises one or more tightly packed colonies of undifferentiated cells, each individual cell of which is smaller than a PGC.

Rather, Appellants respectfully submit that Chang 1997 teaches the short-term (*i.e.*, 5 day) culture of chicken PGCs *per se*. Chang 1997 does not disclose that at 5 days, colonies of undifferentiated chicken cells are produced. This is clearly pointed out on page 496 of Chang 1997, wherein it is states that "[a]fter 5 days in culture, the gPGCs landing on primary cultured GRSCs were suspended by gentle pipetting without using digestive enzymes". Chang 1997 at page 496 (emphasis added). As one of ordinary skill in the art would recognize, "gentle pipetting without the use of digestive

enzymes" would not release colonies of undifferentiated cells, which require the use of digestive enzymes to disrupt the colonies into individual cells.

Thus, it is respectfully submitted that the cells disclosed in Chang 1997 are simply PGCs themselves. Accordingly, like Chang 1995, Chang 1997 only discloses expanding PGC numbers in culture and that when the PGCs are reintroduced into avian embryos they behave just like normal PGCs – they colonize the gonad. Stated another way, Chang 1997 teaches a method of culturing PGCs to produce an increased number of PGCs. This is unlike the sustained culture of claim 44, which comprises undifferentiated chicken cells, wherein the undifferentiated chicken cells (i) are derived from chicken PGCs isolated from the genital ridge or gonad of a later than stage 14 embryo, (ii) are smaller than the chicken PGCs so isolated; and (iii) form one or more colonies of tightly packed undifferentiated chicken cells that express an embryonic stem cell phenotype.

Since Chang 1997 does not disclose each and every element of claim 44, Appellants respectfully submit that Chang 1997 does not anticipate claim 44. Claims 47, 48, 52-54, and 58 all depend directly or indirectly from claim 44, and thus include all the elements of distinguished claim 44. Accordingly, Appellants respectfully request that the rejection of claims 44, 47, 48, 52-54, and 58 in view of Chang 1997 be reversed.

Additionally, Appellants respectfully submit that Chang 1997 does not disclose the elements recited in dependent claims 53, 54, and 58. Particularly, Appellants respectfully submit that since Chang 1997 does not disclose maintaining the undifferentiated state for at least one (claim 53) or two (claim 54) months or maintaining the embryonic stem cell phenotype when grown on the preconditioned fibroblast feeder matrix in the presence of the conditioned media for at least three days (claim 58).

Therefore, while claims 53, 54, and 58 are believed to be distinguished over Chang 1997 by virtue of their dependence from distinguished claim 44, these claims are also believed to be distinguished from this reference as a result of the additional elements recited in the claims. As such, Appellants respectfully submit that claims 53, 54, and 58 are all independently distinguishable over Chang 1997.

F. Rejection under 35 U.S.C. § 102(e) over any of the '740 Patent, the '479 Patent, and the '510 Patent (Claims 44, 47, 48, 51-54, and 56-58)

Claims 44, 47, 48, 51-54, and 56-58 have been rejected under 102(e) as being anticipated by U.S. Patent Nos. 5,340,740; 5,656,479; or 5,830,510; all to Petitte *et al.* (hereinafter "the '740 Patent", "the '479 Patent", and "the '510 Patent", respectively, and hereinafter collectively referred to as "the Petitte Patents"). According to the Examiner, the Petitte Patents teach culturing cells from a stage X embryo and isolating PGCs ('740 Patent). The cells were seeded onto chicken embryonic fibroblast feeder layers and cultured with BRL conditioned medium. The Examiner asserted that "PGCs isolated from stage X are equivalent to PGCs isolated later than stage 14 as claimed because PGCs isolated from stage X and XIV have the same function". *See* Official Action dated April 6, 2005, at page 19.

Appellants respectfully traverse the instant rejection. Appellants respectfully submit that the Petitte Patents do not disclose each and every element of the present claims. Specifically, the Petitte Patents do not disclose the use of PGCs isolated from the gonad or genital ridge of an avian embryo at a stage later than stage 14.

Appellants respectfully submit that the Examiner's assertion that the Petitte Patents disclose that "PGCs and stromal cells were inherently 'isolated together from the embryonic genital ridge or gonad' as claimed because the whole embryo was isolated and inherently contained both PGCs and stromal cells in the genital ridge or gonad" is scientifically inaccurate because stage X embryos have neither a genital ridge nor a gonad. The chicken embryo at Stage X (*i.e.*, a blastoderm stage) has not yet formed the three primary germ layers: ectoderm, mesoderm, and endoderm. Hence, the Examiner's assertion that genital ridge and/or gonadal stromal cells exist in a Stage X embryo is scientifically inaccurate. Thus, Appellants respectfully submit that one of ordinary skill in the art would understand that those structures are not present in the Stage X embryo. Accordingly, the Petitte Patents cannot be read to disclose this element of claim 44.

Since the Petitte Patents do not disclose each and every element of claim 44, Appellants respectfully submit that the Petitte Patents do not anticipate claim 44. Claims 47, 48, 51-54, and 56-58 all depend directly or indirectly from claim 44, and thus include all the elements of distinguished claim 44. Accordingly, Appellants respectfully request the reversal of the rejection of claims 44, 47, 48, 52-54, and 56-58 in view of the Petitte Patents.

Additionally, Appellants respectfully submit that the Petitte Patents do not disclose the elements recited in dependent claims 47 and 48. Particularly, Appellants respectfully submit that the Petitte Patents do not disclose a preconditioned feeder matrix comprising cells that have been isolated from the gonad (claim 47) or genital ridge (claim 48) of a chicken embryo later than stage 14.

Therefore, while claims 47 and 48 are believed to be distinguished over the Petitte Patents by virtue of their dependence from distinguished claim 44, these claims are also believed to be distinguished from this reference as a result of the additional elements recited in the claims. As such, Appellants respectfully submit that claims 47 and 48 are all independently distinguishable over the Petitte Patents.

G. Rejection under 35 U.S.C. § 103(a) over U.S. Patent No. 6,156,569 to Ponce de Leon in view of Chang 1995 (Claims 44, 47, 48, 51-54, and 56-58)

Claims 44, 47, 48, 51-54, and 56-58 have been rejected under 35 U.S.C. § 103(a) upon the contention that the claims are obvious over U.S. Patent No. 6,156,569 to Ponce de Leon (hereinafter "the '569 Patent") in view of Chang 1995.

Appellants respectfully disagree. Appellants respectfully direct the Board's attention to the comments presented hereinabove with respect to Chang 1995. Particularly, Appellants respectfully submit that Chang 1995 does not disclose PGC-derived cells that are smaller than primordial germ cells and that form one or more colonies of tightly packed undifferentiated avian cells as recited in claim 44.

Additionally, Appellants respectfully submit that this deficiency is not cured by the '569 Patent, which also discloses a long-term culture system for PGCs. Thus, neither reference individually, nor the combination of references, discloses or suggests a

sustained culture as claimed in claim 44 because there is no disclosure of PGC-derived cells that form one or more colonies of tightly packed undifferentiated chicken cells that are smaller than primordial germ cells.

Appellants respectfully submit that all of the Examiner's assertions with regard to the instant rejection appear to be based on the contention that the undifferentiated chicken cells claimed in the instant application are PGCs, or alternatively, that the claimed cells are not patentably distinct from PGCs. Appellants respectfully submit that neither of these two assertions is accurate. First, the claimed cultures comprise derivatives of PGCs. This is clearly pointed out in the express language of claim 44, which recites that the one or more colonies comprise tightly packed undifferentiated chicken cells that are smaller than primordial germ cells. Additionally, since the claimed cells are structurally distinct from PGCs, the Examiner's assertion that the claimed undifferentiated cells and PGCs are structurally and functionally the same is also believed to be inaccurate.

To elaborate, Appellants respectfully submit that at best the '569 Patent teaches a method of long term culturing of PGCs *per se* in a feeder-free culture with the addition of exogenous growth factors including LIF, bFGF, IGF, and SCF (*see* col. 5, lines 20-50). Appellants respectfully submit that there is no disclosure in the '569 Patent of any morphological change of the PGCs in the culture to cells that are smaller than PGCs and form tightly packed colonies as recited in claim 44 of the instant application.

Furthermore, Appellants respectfully submit that there is no disclosure in the '569 Patent of making somatic chimeras using PGCs. Rather, the '569 Patent discloses the following:

> As discussed, these PGCs can be maintained for long periods in culture with the successful production of chimeric avians. To date, the cells have been maintained in tissue culture for up to about 4 months, with apparently no adverse effects. Also, cells of up to 25 days have been tested for their ability to effectively colonize avian embryonic gonads and produce chimeric birds. However, it is expected that these cells can be cultured indefinitely, with retention of the ability to produce chimeric birds.
>
> Methods for using PGCs to produce chimeras are known in the art as evidenced by the prior art discussed supra. Preferably, PGCs will be transferred into recipient avian embryos according to the methods

> disclosed in the example while follows. Thereafter, successful chimera production is evaluated based on migration and colonization of PGCs in the gonads, retention of PGC phenotype, or by evaluating for the presence of donor PGCs in gonads after hatching and breeding.
>
> In the present example, the inventors selected genotypes which are easily followed which affect coloration. Donor birds were white broiler type and recipient birds were black feathered birds, respectively, having specific potential genotypes. The putative chimeras were black feathered and produced black/white progeny when mated with black birds. Thereby, successful chimeras were demonstrated based on the production of black/white feathered progeny produced after mating the putative chimeric bird with another black feathered bird.

'569 Patent at column 6, lines 21-48 (emphasis added). Appellants respectfully submit that it is clear from this passage that if chimeras are produced by re-introducing cultured PGCs into an embryo, the chimeras were germline chimeras only. This is shown by the disclosure that all the putative chimeras were black feathered (*i.e.*, no PGC contribution to the feathers in the chimeras) but produced some black/white feathered progeny (*i.e.*, there was PGC contribution to the germline).

Thus, even assuming *arguendo* that the '569 Patent discloses the production of chimeric avians, Appellants respectfully submit that it discloses the production of only germline chimeric avians. Appellants respectfully submit that since PGCs differentiate into the germ cells, successful colonization of the gonad by PGCs introduced into an avian embryo would be expected to produce germline chimeric birds. Again, however, the instantly claimed cultures are not cultures of PGCs, but are sustained cultures of cells that are derivatives of PGCs. As such, the '569 Patent does not support the instant rejection.

Turning now to the disclosure of Chang 1995, Appellants respectfully submit that the comments presented hereinabove with respect to the '569 Patent are equally applicable to Chang 1995. Indeed, the disclosure of Chang 1995 does not support the deficiencies of the '569 Patent in the cited combination. The Board's attention is also directed to Figure 2 of Chang 1995, which shows PGCs growing in culture. The Figure legend clearly indicates that PGCs grew individually or as aggregates, which Appellants respectfully submit are not tightly packed colonies of cells that are smaller than PGCs

as recited in claim 44. In particular, Figure 2(d) of Chang 1995 shows an aggregate that is considerably different morphologically from the colonies of undifferentiated cells disclosed in the instant application (*compare* Figure 4 of the instant application). *See also* the Petitte Declaration, Point 12).

Accordingly, Appellants respectfully submit that the combination of the '569 Patent and Chang 1995 does not support the instant rejection of claims 44, 47, 48, 51-54, and 56-58 under § 103(a), and respectfully request that the rejection of claims 44, 47, 48, 51-54, and 56-58 be reversed.

Additionally, Appellants respectfully submit that claims 47, 48, 51, 53, 54, and 56-58 are each independently distinguishable from the cited combination of the '569 Patent and Chang 1995. With respect to claims 47 and 48, Appellants respectfully submit that the combination of the '569 Patent and Chang 1995 does not render obvious the use of a preconditioned feeder matrix comprising cells that have been isolated from the gonad (claim 47) or the genital ridge (claim 48) of a chicken embryo later than stage 14 according to the Hamburger & Hamilton staging system. While the '569 Patent might disclose the use of avian fibroblasts as feeder cells, the '569 Patent explicitly discloses: 'In particular, the use of fibroblasts, preferably avian fibroblasts, and most preferably Gallinacea fibroblasts (still more preferably chicken fibroblasts), will provide for maintenance of PGCs in tissue culture provided that the four essential growth factors are present" (*see* column 5, lines 50-54; emphasis added). Appellants respectfully submit that claims 47 and 48 do not require the present of the "four essential growth factors" (*i.e.*, LIF, bFGF, SCF, and IGF), and thus the disclosure of the '569 Patent teaches away from the generalized use of chicken fibroblasts as a feeder layer.

Turning now to claim 51, Appellants respectfully submit that neither the '569 Patent nor Chang 1995 discloses the use of BRL-conditioned medium to maintain PGC-derived cells in an undifferentiated state. Therefore, one of ordinary skill in the art would have found no motivation in the combination of the '569 Patent and Chang 1995 to use BRL-conditioned medium. Thus, Appellants respectfully submit that claim 51 is independently patentable over the combination of the '569 Patent and Chang 1995.

With respect to claims 53, 54, and 58, Appellants respectfully submit that the combination of the '569 Patent and Chang 1995 do not relate to maintenance of PGC-derived cells that express an ES cell phenotype, and thus cannot be read to motivate one of ordinary skill in the art to produce cultures of such cells that maintain the undifferentiated state for at least one (claim 53) or two (claim 54) months, or when grown on a preconditioned fibroblast feeder matrix in the presence of the conditioned media for at least three days (claim 58). Thus, Appellants respectfully submit that claims 53, 54, and 58 are also independently patentable over the combination of the '569 Patent and Chang 1995.

And finally, Appellants respectfully submit that the combination of the '569 Patent and Chang 1995 do not disclose or suggest the use of mouse fibroblasts (*e.g.*, STO cells) as a feeder layer. Chang 1995 discloses the use of avian fibroblasts, and the '569 Patent suggests that avian feeders are preferable but only if LIF, bFGF, SCF, and IGF are also present. Thus, Appellants respectfully submit that one of ordinary skill in the art would not have been motivated to prepare the sustained cultures of claims 56 and 57 after consideration of the '569 Patent and Chang 1995.

Summarily, Appellants respectfully submit that the elements recited in claims 47, 48, 51, 53, 54, and 56-58 provide independent bases for distinguishing these claims over the cited combination of the '569 Patent and Chang 1995.

H. Rejection under the Judicially-created Doctrine of Obviousness-type Double Patenting over claims 1 and 8-10 of the '740 Patent in view of the disclosure of the '740 Patent and Chang 1995 (Claims 44, 47, 48, 51-54, and 56-58)

Claims 44, 47, 48, 51-54, and 56-58 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 8-10 of the '740 Patent in view of Chang 1995. The assertions upon which the instant rejection is based can be found on pages 17-20 of the Final Official Action.

Appellants respectfully submit that the combination of the '740 Patent and Chang 1995 does not support a *prima facie* case of obviousness of claims 44, 47, 48, 51-54,

and 56-58, and thus cannot support the instant obviousness-type double patenting rejection. Appellants respectfully submit that the combination of the '740 Patent and Chang 1995 does not provide the requisite motivation for one of ordinary skill in the art to produce the claimed sustained cultures, does not provide a reasonable expectation that the claimed sustained culture could indeed be produced, nor do the references individually or in combination disclose or suggest each and every element of the claims as is required under M.P.E.P. § 804.

To elaborate, M.P.E.P. § 804 states that "the analysis employed in an obviousness-type double patenting determination parallels the guidelines for a 35 U.S.C. 103(a) rejection, the factual inquiries set forth in *Graham v. John Deere Co.,* 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are employed when making an obvious-type double patenting analysis". Accordingly, it is clear that the Examiner must establish a *prima facie* case of obviousness of the rejected claims, which would include a showing consistent with the requirements of M.P.E.P. § 2143 (*i.e.*, there must be some suggestion or motivation to modify the reference or to combine reference teachings; there must be a reasonable expectation of success; and the prior art reference or references when combined must teach or suggest all the claim limitations).

Appellants respectfully submit that a *prima facie* case of obviousness has not been established in that one of ordinary skill in the art would not have been motivated to employ PGCs isolated from the genital ridge or gonad of a later than stage 14 chicken in the methods of the '740 Patent, even in view of Chang 1995. Additionally, a reasonable expectation of successfully doing so has not been established, nohas it been established that the combination of the '740 Patent and Chang 1995 teaches or suggests each and every element of the presently rejected claims.

Particularly, Appellants respectfully submit that claims 1 and 8-10 of the '740 Patent, either alone or in combination with Chang 1995, would not have motivated one of ordinary skill in the art to employ PGCs isolated from the genital ridge or gonad of a later than stage 14 chicken in the claimed methods. Appellants respectfully submit that no such motivation to do so has been provided. The only motivation ever asserted in

the prosecution of the instant application related to the use of avian feeder cells, which Chang 1995 is asserted to teach can be used to increase the number of PGCs in the culture (*see* Final Official Action dated May 31, 2002 at page 14).

In response to this assertion, Appellants argued in the After Final Amendment dated November 27, 2002 that the combination of the '740 Patent and Chang 1995 does not disclose the use of PGCs isolated from an avian embryo later than stage 14 to form a sustained culture comprising undifferentiated avian cells expressing an embryonic stem cell phenotype as recited in claim 44. In response, the Examiner asserted that the stage of isolation of the PGCs does not bear patentable weight because the product claimed has the same structure and function as that described in the '740 Patent.

Appellants respectfully disagree. Appellants respectfully submit that in order to establish a *prima facie* case of obviousness, a showing must be provided that that one of ordinary skill in the art would have been motivated to produce the instantly claimed cultures as of the filing date of the instant application. Appellants respectfully submit that the Examiner has not provided this showing. Further, Appellants respectfully submit that it would not be possible to show such motivation because the understanding of the ordinary artisan at the time the application was filed was that PGCs isolated after stage 14 would be incapable of forming the claimed sustained culture.

To elaborate, Appellants respectfully submit that the subject matter of claims 44 and dependent thereof relates to a combination of a preconditioned feeder matrix, conditioned medium, and chicken primordial germ cells and stromal cells, wherein the chicken primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system, and grown in the sustained culture to produce undifferentiated chicken cells expressing an embryonic stem cell phenotype. Thus, the PGCs so isolated have migrated to and localized in the embryonic genital ridge or gonad.

Appellants respectfully submit that the presence of the PGCs in the embryonic genital ridge or gonad is of considerable relevance to the question of whether one of ordinary skill in the art would have been motivated to use these particular cells.

Appellants respectfully submit that it was the understanding of the skilled artisan at the time of filing of the instant application that PGCs present in the genital ridge/gonad microenvironment were destined for terminal differentiation into germ cells.

Appellants respectfully direct the Board's attention to page 10, line 22, through page 11, line 2, of the instant specification, which states:

> [P]rior to the disclosure of the present invention, it was the general view among those of ordinary skill in the art that avian embryonic gonadal cells comprising primordial germ cells, such as may be collected from, for example, the avian embryonic genital ridge or gonad, once the embryo had reached a stage associated with gonadal development, were to terminally differentiate to germ cells only.

Specification at page 10, line 22, through page 11, line 2 (emphasis added). The "stage associated with gonadal development" corresponds to the arrival of the PGCs into the genital ridge/gonad microenvironment. Thus, Appellants respectfully submit that one of ordinary skill in the art understood prior to the filing of the instant application that once the PGCs localize to the genital ridge/gonad microenvironment, inductive signals generated therein would lead to a restriction in the developmental potential of the PGCs and their derivatives.

As such, appellants respectfully submit that a skilled artisan attempting to produce a culture of undifferentiated cells would not employ cells believed to be committed to terminal differentiation. As such, Appellants respectfully submit that the understanding of the skilled artisan taught away from the use of later than stage 14 PGCs that were isolated from the embryonic genital ridge or gonad. Given this teaching away, Appellants respectfully submit that one of ordinary skill in the art would not have been motivated to employ these cells, and thus the ability of these cells to in fact form undifferentiated avian cells expressing an embryonic stem cell phenotype represents an unexpected result. Therefore, it is only by viewing the prior art with the benefit of hindsight vision gained through Appellants' disclosure that one of ordinary skill in the art would believe that PGCs isolated from the embryonic gonad or genital ridge after stage 14 could form undifferentiated avian cells expressing an embryonic stem cell phenotype.

Summarily, Appellants respectfully submit that there is no motivation in the cited combination or in knowledge of the skilled artisan that would have led one of ordinary skill in the art to use PGCs isolated from the gonad or genital ridge of later than stage 14 embryos to produce sustained cultures of undifferentiated chicken cells as recited in the instant claims. At the time of filing, one of ordinary skill in the art would have believed that the PGCs located in these regions of an embryo at this stage were committed to terminal differentiation and thus unable to generate these cells.

Thus, Appellants respectfully submit that the element "isolated from an embryo later than stage 14" does indeed bear patentable weight in the context of an obviousness-type double patenting rejection because the requisite motivation to combine the references must still be present. Appellants respectfully submit that as with any rejection based on § 103, without a motivation to combine the asserted references, a rejection based on § 103 is improper. Accordingly, the instant rejection should be reversed.

Continuing with the instant rejection, Appellants respectfully submit that the combination of the '740 Patent and Chang 1995 also fails to support the instant rejection because the combination does not disclose or suggest each and every element of the claims. In an attempt to equate certain elements of the instant claims with elements disclosed in the cited references, the Examiner has presented several assertions that Appellants respectfully submit are scientifically inaccurate. For example, the Examiner asserted on page 18 of the Final Official Action that "the cells isolated from Stage X embryos described and claimed in the '740 Patent are equivalent to the undifferentiated cells isolated from a chicken embryo after stage 14 as claimed because both have an ES cell phenotype. Appellants respectfully disagree. As discussed *supra* herein, "ES cell phenotype" relates to a morphological description of the cultured undifferentiated cells that are recognized as being characteristic of ES cells. There is no evidence in the record that any cell that can be isolated from a Stage X blastoderm is morphologically similar to these cells, and thus the Examiner's unsupported assertion is improper.

Additionally, the Examiner asserts that the cells isolated in Example 5 of the '740 Patent inherently comprise stromal cells, which are equivalent to chicken feeder cells as claimed in the instant invention. Appellants respectfully submit that this is also an unsupported assertion that does not rise to the level of "substantial evidence" (*see* M.P.E.P. 2144.03). For example, Appellants respectfully submit that it is at elast equally likely that one of ordinary skill in the art would have believed that the stromal cells co-isolated with PGCs from the genital ridge/gonad microenvironment would produce inductive signals that would push PGCs towards differentiation. Thus, it would not necessarily have been believed that these cells would be appropriate as feeder cells for cells that are intended to be undifferentiated.

Continuing, the Examiner asserted that "there is no apparent reason why applicant was prevented from presenting claims directed toward a culture comprising PGCs and chicken stromal cells isolated together from stage IX-XIV embryos during prosecution of the application that [issued as the '740 Patent]". Appellants respectfully disagree. The application that issued as the '740 Patent was filed in 1992, and as of that date it was not known that PGCs isolated from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14 could give rise to undifferentiated cells, and in fact it was believed that they could not.

Additionally, the '740 Patent does not disclose isolating PGCs and stromal cells from Stage X embryos. The terms "primordial germ cell" and "PGC" do not appear anywhere in the '740 Patent. Rather, the '740 Patent discloses isolating the area pellucida from Stage X embryos (*see* column 7, Example 5, of the '740 Patent).

Further, and as stated hereinabove, one of ordinary skill in the art believed that PGCs later than stage 14 (which Appellants respectfully submit is <u>not</u> a stage that overlaps with "stages IX-XIV"; *see* discussion *supra* regarding the different staging systems) would not have been capable of giving rise to undifferentiated derivatives.

The Examiner also asserts that "the subject matter claimed in the instant application was fully disclosed in the ['740] Patent and was covered by the patent since the patent and the application are claiming common subject matter" (<u>Final Official Action</u> at page 18). This circular reasoning is believed to be clearly in error since claims 1 and

8-10 of the '740 Patent relate to cells isolated from a stage prior to the primitive streak, whereas the instant claims relate to isolating cells from a post-primitive streak stage.

The Examiner next presented an alternative basis for the instant rejection, namely the assertion that if the '740 Patent did not specifically teach a culture comprising PGCs and chicken stromal cells isolated from the germinal ridge of a chicken embryo at a stage later than stage 14, Chang 1995 cured this deficiency. Appellants respectfully disagree. As set forth in more detail hereinabove, Chang 1995 discloses cultures of PGCs *per se*, and not the instantly claimed cultures of derivatives of PGCs. Thus, Chang 1995 does not provide any motivation for one of ordinary skill in the art to employ later than stage 14 PGCs since it provides no suggestion that later than stage 14 PGCs would be able to form undifferentiated cells that would overcome the belief that they would not be appropriate for this purpose.

Turning next to the Examiner's assertion that "the product (stromal cells and cells having an ES cell phenotype) can be isolated from either a whole stage X embryo or the genital ridge of a stage 15 embryo or by mixing PGCs isolated from Stage X embryos with stromal cells isolated from the germinal ridge of Stage XV embryos" on page 19 of the Final Official Action, Appellants respectfully submit that the instant assertion demonstrates a lack of appreciation of the different staging systems that are employed in avian embryology. Appellants respectfully submit that the EGK system employs Roman numerals and the Hamburger and Hamilton (H&H) staging system employs Arabic numerals. As is known in the art, these two staging systems are applicable to mutually exclusive developmental stages, and thus the mixing of "14" with "XIV" and "15" with "XV" is improper.

Appellants further respectfully submit that according to the EGK staging system, there is no Stage XV. The Abstract of the journal article disclosing the EGK system (Hefzibah Eyal-Giladi and Shimshon Kochav (1976) 49 *Developmental Biology* 321-337) states: "Fourteen developmental stages preceding Hamburger and Hamilton's stage 2 have been studied from live material and photographed from both upper and lower surfaces" (emphasis added). Thus, Appellants respectfully submit that there is no Stage XV in chicken development. There is, however, a Stage 15, but that relates to

the Hamburger and Hamilton staging system as used in the instant claims. Thus, Stage XV (if there were such a Stage) and Stage 15 would not be equivalent, and thus, the instant assertion does not support the current rejection.

Continuing with the instant rejection, Appellants respectfully traverse the Examiner's assertion that Appellants have argued that the PGCs of Chang were "terminally differentiated" as contended on page 19 of the Final Official Action. Rather, Appellants have maintained throughout the instant prosecution that the skilled artisan believed at the time instant application was filed that PGCs present within the gonad or genital ridge of later than stage 14 (H&H) embryos were committed to terminal differentiation, not terminally differentiated themselves. Given that the skilled artisan would not have believed that committed cells could form colonies of undifferentiated cells, Appellants respectfully submit that the skilled artisan would not have believed that PGCs isolated from a chicken "later than stage 14 (H&H)" could form colonies of undifferentiated cells.

And finally, Appellants respectfully traverse the Examiner's assertion that "the claims encompass cultures comprising avian cells having any ES cell phenotype" (Final Official Action at pages 19-20, bridging paragraph; emphasis added). Appellants respectfully submit that the phrase "ES cell phenotype" refers to a particular morphological phenotype: namely having "a large nucleus, prominent nucleolus, and little cytoplasm", and that this would be clear to one of ordinary skill in the art upon consideration of the instant specification. The proper framework for determining the scope of claim elements is set forth in *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed.Cir. 2005; *citing Multiform Desiccants, Inc. v. Medzam, Ltd.*, 133 F.3d 1473, 1477 (Fed.Cir. 1998)), which held that "the person of ordinary skill in the art is deemed to read the claim term not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the specification" (*Phillips* at page 1313), Appellants respectfully submit that upon consideration of the specification as a whole, one of ordinary skill in the art would understand the nature of the ES cell phenotype recited in the claims, and that it relates to a morphological phenotype.

Additionally, claim 44 recites *inter alia* that the undifferentiated cells (i) are derived from chicken PGCs isolated from the genital ridge or gonad [of a later than stage 14 embryo], (ii) are smaller than the chicken PGCs [so isolated]; and (iii) form one or more colonies of tightly packed undifferentiated chicken cells expressing an embryonic stem cell phenotype. Thus, the language of claim 44 structurally distinguishes the claimed cells from the PGCs from which they are derived.

Accordingly, Appellants respectfully submit that a *prima facie* case of obviousness has not been made out, and as a result, Appellants respectfully request that the rejection of claims 44, 47, 48, and 51-57 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 8-10 of the '740 Patent in view of the disclosure of the '740 Patent and Chang 1995 be reversed.

I. Rejection under the Judicially-created Doctrine of Obviousness-type Double Patenting over Claim 1 of the '479 Patent or the '510 Patent in view of Chang 1995 (Claims 44, 47, 48, 51-54, 56, and 57)

Claims 44, 47, 48, 51-54, 56, and 57 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of U.S. Patent No. 5,656,479 or 5,830,510 (hereinafter "the '479 Patent" and "the '510 Patent", respectively) in view of Chang 1995. Appellants have carefully considered the rejection and the Examiner's bases therefor, and respectfully traverse the rejection as follows.

Appellants initially note that the discussion presented immediately above with regard to the rejection of claims 44, 47, 48, 51-54, and 56-58 over the '740 Patent in view of the disclosure of the '740 Patent and Chang 1995 is equally applicable to the instant rejection. Summarily, none of the constituent patents that make up the "Petitte Patents" (*i.e.* the '740 Patent, the '479 Patent, and the '510 Patent) disclose the use of PGCs isolated from an avian embryo after stage 14 to produce a culture comprising colonies of undifferentiated chicken cells.

Appellants have contended, and the Examiner has acknowledged, that those of skill in the art believed that the PGCs used to generate the instantly claimed sustained

cultures were believed to be incapable of forming undifferentiated cells expressing an embryonic stem cell phenotype. Thus, the art teaches away from the production of the instantly claimed sustained cultures, and as such, there could have been no motivation to employ PGCs isolated from after stage 14.

As a result, Appellants respectfully submit that one of ordinary skill in the art would find no motivation in the combination of either the '479 Patent or the '510 Patent with Chang 1995 to produce sustained cultures of undifferentiated cells from PGCs isolated from later than stage 14 chicken embryos. Appellants respectfully submit that without such a motivation, the instant rejection is fatally flawed and should be reversed.

The Examiner concedes that the '479 and '510 Patents fail to disclose a culture comprising PGCs and chicken stromal cells isolated from the germinal ridge of a chicken embryo at later than stage 14. The Examiner asserts that Chang 1995 cures the disclosure lacking in the '479 Patent and the '510 Patent. Particularly, the Examiner asserts that:

> Chang taught culturing PGCs with chicken stromal cells isolated from the genital ridge of a stage 27 embryo. Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to isolate chicken cells having an ES cell phenotype as claimed in '479 and '510 wherein the chicken cells are cultured on chicken stromal cells isolated from Stage 27 embryos as taught be Chang. One of ordinary skill in the art at the time the invention was made would have been motivated to use stromal cells isolated from stage 27 chicken embryos to increase the number of PGCs as taught by Chang (abstract).

Final Official Action at pages 20-21 (bridging paragraph).

Appellants first respectfully submit that as used in the instant application and in the claims, the phrase "having an ES cell phenotype" refers to a population of cells that is derived from PGCs, but cannot be "isolated from" a stage 27 embryo as asserted by the Examiner. Appellants respectfully submit that it is known to those of skill in the art that ES cells cannot be "isolated" from any stage of any embryo. Rather, they are produced by culturing certain cell types *in vitro*. As a result, Appellants respectfully submit that the Examiner's assertion that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to isolate avian cells having an ES cell phenotype as claimed in '479 and '510" is scientifically inaccurate.

Furthermore, the proposed motivation to use stromal cells isolated from stage 27 avian embryos that Chang 1995 is asserted to provide would not be relevant to the instantly claimed cultures because the instant claims are not directed to PGC cultures *per se*. Appellants respectfully submit that the increase in PGCs disclosed in Chang 1995 is not shown to be caused by the presence of stage 27 stromal cells, and in fact relates to nothing more than proliferation of the PGCs that are seeded on these cells. Thus, even if stage 27 stromal cells adequately supported the proliferation of PGCs as shown in Chang 1995, the instant claims are not directed to cultures of PGCs. Therefore, one of ordinary skill in the art attempting to produce cultures of undifferentiated cells derived from PGCs would not be interested in producing more PGCs, and in fact might be dissuaded from employing a feeder layer that appears to maintain the PGCs as PGCs.

Accordingly, even assuming *arguendo* that Chang 1995 teaches that culturing PGCs on stage 27 stromal cells would result in PGC proliferation, Appellants respectfully submit that this provides no motivation for employing these cells to produce and maintain cultures of undifferentiated cells derived from PGCs.

Summarily, Appellants respectfully submit that a *prima facie* case of obviousness of claims 44, 47, 48, 51-54, 56, and 57 has not been presented, and thus the instant rejection of these claims under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of either the '479 Patent or the '510 Patent in view of Chang 1995 has not been established. Therefore, the instant rejection should be reversed. Appellants therefore respectfully submit that claims 44, 47, 48, 51-54, 56, and 57 are in condition for allowance.

CONCLUSIONS

For the reasons set forth hereinabove, Appellants respectfully submit that the claims presently pending in the above-captioned application meet of the necessary requirements of patentability. It is therefore respectfully requested that the Board reverse the Examiner and remand this application for issue.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any underpayment or credit any overpayment of fees associated with the filing of this paper to Deposit Account No. **50-0426**.

Respectfully submitted,
JENKINS, WILSON, TAYLOR & HUNT, P.A.

Date: 10/27/2006 By: Arles A. Taylor, Jr.

Arles A. Taylor, Jr.
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297/93/2 AAT/CPP

Customer No: 25297

VIII. Claims Appendix – 37 C.F.R. § 41.37(c)(1)(viii)

44. A sustained culture of undifferentiated chicken cells expressing an embryonic stem cell phenotype, the sustained culture comprising:

(a) a preconditioned feeder matrix;

(b) conditioned media;

(c) chicken primordial germ cells and chicken stromal cells, wherein the chicken primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from a chicken embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system; and

(d) undifferentiated chicken cells expressing an embryonic stem cell phenotype,

wherein the undifferentiated chicken cells:

(i) are derived from the chicken primordial germ cells isolated from the chicken embryo;

(ii) are smaller than the chicken primordial germ cells; and

(iii) form one or more colonies of tightly packed undifferentiated chicken cells expressing an embryonic stem cell phenotype.

47. The sustained culture of claim 44 wherein the preconditioned feeder matrix comprises cells that have been isolated from the gonad of a chicken embryo later than stage 14 according to the Hamburger & Hamilton staging system.

48. The sustained culture of claim 44 wherein the preconditioned feeder matrix comprises cells that have been isolated from the genital ridge of a chicken embryo later than state 14 according to the Hamburger & Hamilton staging system.

51. The sustained culture of claim 44 wherein the conditioned media is Buffalo Rat Liver (BRL) conditioned media.

52. The sustained culture of claim 44 wherein the conditioned media comprises a supplemental growth factor selected from the group consisting of leukemia inhibitory factor (LIF), insulin-like growth factor (IGF), fibroblast growth factor (FGF),

basic fibroblast growth factor (bFGF), stem cell factor (SCF), steel factor (SF), transforming growth factor-β1 (TGF-β1), anti-retinoic acid, and combinations thereof.

53. The sustained culture of claim 44 wherein the embryonic stem cell phenotype is maintained for at least one month.

54. The sustained culture of claim 44 wherein the embryonic stem cell phenotype is maintained for at least two months.

56. The sustained culture of claim 44, wherein the preconditioned feeder matrix comprises mouse fibroblast cells.

57. The sustained culture of claim 56, wherein the mouse fibroblast cells comprise STO cells.

58. The sustained culture of claim 44, wherein the undifferentiated chicken cells maintain the embryonic stem cell phenotype when grown on the preconditioned fibroblast feeder matrix in the presence of the conditioned media for at least three days.

IX. Evidence Appendix – 37 C.F.R. § 41.37(c)(1)(ix)

IX.1. Declaration of James N. Petitte Pursuant to 37 C.F.R. § 1.132

A Declaration of James N. Petitte Pursuant to 37 C.F.R. § 1.132 was submitted on October 6, 2005. The Final Official Action did not address the subject Declaration, although Appellants presume that the subject Declaration was entered into the record. A true and accurate copy of the subject Declaration is attached hereto.

X. Related Proceedings Appendix – 37 C.F.R. § 41.37(c)(1)(x)

There are no appeals or interferences, known to Appellants or Appellants' legal representatives, which will directly affect or be directly affected by or have a bearing on the decision by the Board of Patent Appeals and Interferences (hereinafter "the Board") in the pending appeal.

"Express Mail" mailing number: EV733194025US
Date of Deposit: October 6, 2005
I hereby certify that this paper and all papers and fees referred to herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner of Patents, Washington, D.C.

Amy Yost
Amy Yost

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Petitte *et al.*

Serial No.: 09/757,054

Filed: January 8, 2001

Confirmation No.: 7757

Group Art Unit: 1632

Examiner: Wilson, Michael C.

Docket No.: 297/93/2

For: METHOD OF PRODUCING AN UNDIFFERENTIATED AVIAN CELL CULTURE USING AVIAN PRIMORDIAL GERM CELLS

* *

DECLARATION OF JAMES N. PETITTE, PH.D.
PURSUANT TO 37 C.F.R. §1.132

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. My name is James N. Petitte, Ph.D., and I am Professor of Poultry Science and the Director of the Physiology Graduate Program, at North Carolina State University, assignee for the subject U.S. Patent Application Serial No. 09/757,054.

2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit A**.

3. I have had an opportunity to review pending claims 44, 47, 48, and 51-57 in the above captioned U.S. Patent Application Serial No. 09/757,054.

4. I have also reviewed the following documents: the Official Action dated April 6, 2005 on the above captioned U.S. Patent Application Serial No. 09/757,054 by the U.S. Patent and Trademark Office (USPTO); Chang *et al.* (1995) 19 *Cell Biol Intl* 143-149 (hereinafter "Chang 1995"); Chang *et al.* (1997) 21 *Cell Biol Intl* 495-499 (hereinafter "Chang 1997"); Ponce de Leon *et al.* (1997) 21 *Revista Brasileira de Reproducao Animal* 96-101 (hereinafter "Ponce de Leon") and U.S. Patent No. 6,156,569 to Ponce del Leon *et al.* (hereinafter "the '569 Patent").

5. The subject matter of the pending claims relates to a sustained culture of undifferentiated avian (*e.g.*, chicken) cells. The culture is produced by plating primordial germ cells (PGCs) isolated from the genital ridge or gonad of a post-stage 14 avian embryo on a preconditioned feeder matrix and growing the cells, which are co-isolated with stromal cells, in the presence of conditioned medium. Colonies of undifferentiated cells derived from the PGCs arise. These colonies are tightly packed and have well-defined colony borders as shown in Figure 4D of the above captioned U.S. Patent Application Serial No. 09/757,054.

6. The undifferentiated cells of the above captioned U.S. Patent Application Serial No. 09/757,054 are not PGCs, but are derived from PGCs. The undifferentiated cells have an embryonic stem (ES) cell morphology. They are smaller than the PGCs from which they are derived, and have a large nucleus, a prominent nucleolus, and relatively little cytoplasm (*i.e.*, have a high nucleus:cytoplasm ratio).

7. This morphological description of the undifferentiated cells has been used in the art for many years. For example, several patents issued to me as a co-inventor employ this descriptive terminology. U.S. Patent No. 5,340,740 states: "The established ESC lines from mouse embryos have a characteristic phenotype consisting of a large nucleus, a prominent nucleolus, and relatively little cytoplasm" (*see* column 1, lines 21-24). *See also* U.S. Patent No. 5,656,479 at column 1, lines 18-21; and U.S. Patent No. 5,830,510 at column 1, lines 22-25.

8. PGCs, in contrast to the undifferentiated cells of the claimed sustained cultures, are larger, have a lower nucleus:cytoplasm ratio, and grow individually or in loose aggregates in culture.

9. The presently claimed undifferentiated avian cells are derived from PGCs, but are not themselves PGCs or some fractional component of a population of cells isolated from the gonad or genital ridge.

10. The Patent Office's attempt to functionally define ES cells as cells that are able to contribute to the germline and somatic lineages is both over broad and too narrow. As to the former, a spermatozoan and an oocyte can give rise to all germline and somatic lineages, but one of ordinary skill in the art would not consider either to be an ES cell. With regard to the latter, there are ES cell lines available that do not give rise to germline chimeras, yet are nonetheless considered ES cells. These cell lines can still give rise to many if not all somatic lineages, and as such still can be used in the study of developmental biology and to express transgenes in these tissues.

11. Introduction of PGCs into recipient embryos results in the PGCs homing to the gonad of the embryo, where it takes part in normal gonadal development. When the embryo is born, it may be a germline chimera (dependent on the successful colonization of the gonad by the transferred PGCs), but it will not be a somatic chimera. This is shown in the '569 Patent, where all of the recipients had black feathers (*i.e.*, no PGCs contribution to the soma), but some of the offspring of the recipients had white feathers. Additionally, the Ponce de Leon journal article refers to the recipients as "putative chimeric chickens", and the authors have to breed the putative chimeras to determine whether or not they are in fact chimeric. The breeding was required because the authors were concerned with germline chimerism, which is the only chimerism that can result from the transfer of PGCs. As a result, the Patent Office's assertion on page 8 of the Official Action that "the PGCs of Ponce de Leon are ES cells because they provide

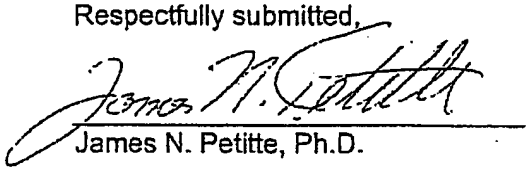
germ and somatic cell chimeras upon being introduced into recipient embryos" is not believed to be accurate.

12. PGCs do not form colonies when cultured *in vitro*. At best, PGCs in culture form loose aggregates.

13. With regard to the differences between PGCs isolated from stage 14 (Hamburger and Hamilton staging system; hereinafter "H&H") or earlier embryos versus PGCs isolated from the genital ridge or gonad of later than stage 14 embryos, prior to the instant disclosure, it was believed in the art that PGCs that had migrated to the gonad or genital ridge were committed to terminal differentiation. PGCs can be found in the genital ridge and/or gonad of later than stage 14 (H&H) chicken embryos.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,



James N. Petitte, Ph.D.

10/5/05

Date

Attachment: **Exhibit A**

Exhibit A

CURRICULUM VITAE of James N. Petitte, Ph.D.

CURRICULUM VITAE

James N. Petitte

Office:
Department of Poultry Science
North Carolina State University
Box 7608
Raleigh, NC 27695-7608
919-515-5389/ j_petitte@ncsu.edu
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Home:
620 Shadywood Lane
Raleigh, NC 27603
919-779-5032

EDUCATION

Ph.D., Major: Reproductive Physiology, Minors: Endocrinology and Animal Breeding, 1986, University of Guelph, Guelph Ontario, Canada. (Thesis title: The influence of the adrenal gland on reproductive function and the timing of ovulation in the domestic hen.)

M.S., Animal Sciences, 1981, University of Maine, Orono, Maine. (Thesis title: Studies on the reproductive performance of caged broiler breeders.)

A.B., Biology, 1979, A.B. (magna cum laude), Susquehanna University, Selinsgrove, PA.

PROFESSIONAL EXPERIENCE

2001-Present	**Professor,** Department of Poultry Science, North Carolina State University, Raleigh, NC
2000-Present	**Director,** Physiology Graduate Program, College of Agriculture and Life Sciences and College of Veterinary Medicine, North Carolina State University, Raleigh, NC
1996-2000	**Associate Professor,** Department of Poultry Science, North Carolina State University, Raleigh, NC
1990-1996	**Assistant Professor,** Department of Poultry Science, North Carolina State University, Raleigh, NC
1989-1990	**Research Associate,** Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1989-1990	**Special Graduate Faculty,** Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1986-1988	**Postdoctoral Fellow,** Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1982-1984	**Graduate Teaching Assistant,** Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1981-1982	**Instructor,** Department of Animal and Veterinary Sciences, University of Maine, Orono, Maine
1979-1981	**Graduate Teaching Assistant,** Department of Animal and Veterinary Sciences, University of Maine, Orono, Maine

SCHOLARLY AND PROFESSIONAL HONORS

Poultry Science Research Award (1997)
U.S.A. Branch World's Poultry Science Travel grant (1992) to present a presentation at the World's Poultry Congress, Amsterdam.
Canadian Branch World's Poultry Science travel grant (1984) to present a poster at the World's Poultry Congress, Helsinki
Taffy Davison Memorial Research Travel Grant (1984) University of Guelph Visa Scholarship (1985) awarded to outstanding non-Canadian graduate students.
James A. McGrath Memorial Fellowship, Department Animal and Poultry Science, University of Guelph (1983)
University Graduate Scholarship, The Graduate School, University of Guelph (1983).
University Graduate Fellowship, The Graduate School, University of Guelph (1982).
Hubbard Farms Scholarship, University of Guelph (1982) and University of Maine (1979, 1980).
Beta Beta Beta Biological Honor Society, Susquehanna University (1978).

PROFESSIONAL SOCIETY MEMBERSHIPS

1979 - Present	Poultry Science Association
1983 - Present	World's Poultry Science Association
1982 - Present	Society for the Study of Reproduction
1990 - Present	American Association for the Advancement of Science
1990 - Present	North Carolina Poultry Federation
1990 - Present	Triangle Reproductive Biologists
1991 - Present	Triangle Transgenic Group
1994 - 1996	Society for In Vitro Biology
1996 - Present	Society for Developmental Biology
1996 - Present	Federation of American Societies for Experimental Biology

PUBLICATIONS (Refereed Journals)

1. Mozdziak, P.E., Q. Wu, J.M. Bradford, S. L. Pardue, C. Giamario, S. Borwornpinyo, and **J. N. Petitte.** 2005. Identification of lacZ insertion site and beta-Galactosidase expression in transgenic chickens. Cell and Tissue Research, in press.

2. Borwornpinyo, S., J. Brake, P. E. Mozdziak, and J. N. Petitte. 2005. Culture of chicken embryos in surrogate eggshells. Poultry Science 84:1477-1482.

3. Mozdziak, P.E., J. Angerman-Stewart, B. Rushton, S. L. Pardue, and **J. N. Petitte**. 2005. Isolation of chicken primordial germ cells using fluorescence-activated cell sorting. Poul. Sci. 84:594-600.

4. Song, Y., S. D'Costa, S. L. Pardue, and **J. N. Petitte**. 2005. Production of germline chimeric chickens following the administration of a busulfan emulsion. Molecular Reproduction and Development. 70:438-444.

5. Mozdziak, P. E., **J. N. Petitte,** and S. D. Carson, 2004. An introductory undergraduate course covering animal cell culture techniques. Biochemistry and Molecular Biology Education 32: 319-322

6. **Petitte, J. N.**, G. Liu, and Z. Yang. 2004. Avian pluripotent stem cells. Mechanisms of Development. 121(9): 1159-1168.

7. Mozdziak, P. E. and **J. N. Petitte**, 2004. Status of transgenic chicken models for developmental biology. Developmental Dynamics. 229(3): 414-421.

8. Mozdziak, P. E., S. Pophal, S. Borwornpinyo, and **J. N. Petitte**. 2003. Transgenic chickens expressing β-galactosidase hydrolyze lactose in the intestine. Journal of Nutrition. 133: 3076-3079.

9. Mozdziak, P. E., S. Borwornpinyo, D. W. McCoy, **J. N. Petitte**. 2003. Development of transgenic chickens expressing bacterial beta-galactosidase. Development Dynamics. 226: 439-445.

10. Giamario, C., **J. N. Petitte**, P. E. Mozdziak. 2003. Hatchability of chicken embryos following somite manipulation. BioTechniques. 34: 1128-1130.

11. Pardue, S.L., D'Costa, S., Song, Y and **Petitte, J.N.**, 2002. Production of inter- and intra-specific germline chimeras in poultry: potential applications. Australian Poultry Science. 14:54-60.

12. **Petitte, J.N.** 2002. The avian germline and strategies for the production of transgenic poultry. Journal of Poultry Science, 39: 205-228.

13. D'Costa, S., S.L. Pardue, and **J.N. Petitte,** 2001. Comparative Development of Avian Primordial Germ Cells and Production of Germ Line Chimeras. Poultry and Avian Biology Reviews. 12 (4): 151-168

14. Fasenko, G. M., V. L. Christensen, M. J. Wineland, and **J. N. Petitte**. 2001. Examining the effects of pre-storage incubation of turkey breeder egg on embryonic development and hatchability of eggs stored for four or fourteen days. Poul. Sci. 80 (2): 132-138.

15. Nolan, C. M., Killian, J. K., **Petitte, J. N.** and R. L. Jirtle. 2001. Imprint Status of M6P/IGF2R and IGF2 in Chickens. Development, Genes and Evolution. 211 (4): 179-183.

16. L. Karagenç and **J. N. Petitte**. 2000. Soluble factors and the emergence of chick primordial germ cells *in vitro*. Poul. Sci. 79:80-85.

17. D'Costa, S., M. J. Kulik, and **J. N. Petitte**. 2000. Expression and purification of biologically active recombinant quail stem cell factor in *E. coli*. Cell Biology International 24:311-317.

18. D'Costa, S. and **J. N. Petitte**. 1999. Characterization of stage-specific embryonic antigen-1 (SSEA-1) expression during early development of the turkey embryo. International Journal of Developmental Biology 43:349-356.

19. D'Costa, S. and **J.N. Petitte**. 1998. Rapid sex determination of turkeys using multiplex PCR. Poultry Science 77:718-721.

20. **Petitte, J.N.**, L. Karagenç, and M. Ginsburg. 1997. The origin of avian primordial germ cells and transgenesis in poultry. Poultry Science 76: 1084-1092.

21. Brake, J.T., T.J. Walsh, C.E. Benton, Jr., **J.N. Petitte**, R. Meijerhof, and G. Penalva. 1997. Egg handing and storage. Poultry Science 76: 144-151.

22. **Petitte, J.N.** and L. Karagenç. 1996. Growth factors during early events in avian embryo development. Poultry and Avian Biology Reviews 7:75-87.

23. Karagenç, L., Y. Cinnamon, M. Ginsburg, and **J. N. Petitte**. 1996. The origin of avian primordial germ cells in the prestreak embryo. Developmental Genetics 19:290-301.

24. **Petitte, J.N.** and M. J. Kulik. 1996. Cloning and characterization of cDNAs encoding two forms of avian stem cell factor. Biochimica Biophysica Acta 1307:149-151.

25. **Petitte, J.N.** and L. Karagenç. 1996. Growth factors during early events in embryo development. Poultry Avian Biol. Rev. 7:5-87.

26. **Petitte, J.N.**, J.M. Petitte and S. Scheideler. 1996. Determination of genetic diversity in commercial ratite stocks using multilocus DNA fingerprinting. Pages 69-77 in "Improving our understanding of ratites in a farming environment", ed. Deeming, ed., Ratite Conference: Banbury

27. Nicolas-Bolnet, C., P.A. Johnston, A.E. Kemper, C. Ricks, and **J.N. Petitte**. 1995. Synergistic action of two sources of avian growth factors on proliferative differentiation of chick embryonic hematopoietic cells. Poultry Science 74: 1102-1116.

28. **Petitte, J.N.** and A. E. Kegelmeyer. 1995. Rapid sex determination of chick embryos using the polymerase chain reaction. Animal Biotechnology, 6:119-130.

29. Yang, Z. and **J.N. Petitte**. 1994. Use of avian cytokines in mammalian embryonic stem cell culture. Poultry Science 73:965-974.

30. **Petitte, J.N.**, M. Kulik, and A.E. Kegelmeyer. 1994. Genomic DNA extraction from avian whole blood. Biotechniques 17: 664-666.

31. Qureshi, M.A., J.A. Marsh, R.R. Dietert, Y-J. Sung, C. Nicolas-Bolnet, and **J.N. Petitte**. 1994. Profiles of chicken macrophage effector functions. Poultry Science 73:1027-1034.

32. Millam, J.R., C.B. Craig-Veit, and **J.N. Petitte**. 1993. Brain content of cGn-RH I and II during embryonic development in chickens. General and Comparative Endocrinology 92: 311-317.

33. Qureshi, M.A., **J.N. Petitte**, S.M. Laster, and R.R. Dietert. 1993. Avian macrophages: contribution to cellular microenvironment and changes in effector functions following activation. Poultry Science 72:1280-1284.

34. Watt, J.M., **J.N. Petitte**, and R.J. Etches. 1993. Early development of the chick embryo. Journal of Morphology 215:165-182.

35. **Petitte, J.N.**, C.L. Brazolot, M.E. Clark, G. Liu, A.M. Verrinder Gibbins, and R.J. Etches. 1993. Accessing the genome of the chicken using germline chimeras. In: Manipulation of the Avian Genome, R.J. Etches and A.M.V. Gibbins, eds., CRC, Ann Arbor.

36. Havenstein, G.B., L.B. Crittenden, **J.N. Petitte**, M.A. Qureshi, and D.N. Foster. 1992. Application of biotechnology to the poultry industry. Animal Biotechnology 3: 15-36.

37. Brazolot, C., **J.N. Petitte**, R.J. Etches, and A.M. Verrinder Gibbins. 1991. Efficient transfection of chicken cells by lipofection and introduction of transfected blastodermal cells into the embryo. Molecular Reproduction and Development, 30:304-312.

38. **Petitte, J.N.**, M.E. Clark, and R.J. Etches. 1991. Assessment of functional gametes in chickens following primordial germ cell transfer. Journal Reprod. and Fert. 92:225-229.

39. **Petitte, J.N.** and R.J. Etches. 1991. Daily infusion of corticosterone and reproductive function in the domestic hen (*Gallus domesticus*). General and Comparative Endocrinology 83:397-405.

40. **Petitte, J.N.**, R.J. Etches, and C.E. Anderson Langmuir. 1991. The effect of metyrapone on the timing of oviposition and ovarian steroidogenesis the laying hen. British Poultry Science 32:809-819.

41. Fasenko, G.M., F.E. Robinson, J.G. Armstrong, J.S. Church, R.T. Hardin, and **J.N. Petitte**. 1991. Variability in preincubation embryo development in domestic fowl. 1. Effects of nest holding time and method of egg storage. Poultry Sci. 70:1879-1881.

42. Etches, R.J. and **J.N. Petitte**. 1990. Avian and reptilian follicular hierarchies-models for ovarian development. Journal of Experimental Zoology S4:112-122.

43. **Petitte, J.N.,** M.E. Clark, G. Liu, A.M. Verrinder Gibbins and R.J. Etches. 1990. The production of somatic and germline chimeras in the chick by transfer of early blastodermal cell. Development 108: 185-190.

44. **Petitte, J.N.** and R.J. Etches. 1989. The effect of corticosterone on the response of the ovary to pregnant mare's serum gonadotrophin in sexually immature pullets. General and Comparative Endocrinology 74: 377-384.

45. **Petitte, J.N.** and R.J. Etches. 1988. The effect of corticosterone on the photoperiodic response of sexually immature fowl. General and Comparative Endocrinology 69: 424-430.

46. Etches, R.J., **J.N. Petitte** and C. Anderson-Langmuir. 1984. Interrelationships between the hypothalamus, pituitary gland, ovary, adrenal gland and the open period for LH release in the hen (Gallus domesticus). Journal of Experimental Zoology 232: 501-511.

47. **Petitte, J.N.,** R.J. Etches, and J.S. Walton. 1984. Normal sexual maturation and reproductive function in domestic hens following unilateral adrenalectomy. Domestic Animal Endocrinology 1: 189-198.

48. **Petitte, J.N.,** R.O. Hawes, and R.W. Gerry. 1983. The influence of cage vs. floor pen management of broiler breeder hens on subsequent performance of cage reared broilers. Poultry Science 62: 1241-1246.

49. **Petitte, J.N.,** R.O. Hawes, and R.W. Gerry. 1982. The influence of flock uniformity on the reproductive performance of broiler breeder hens housed in cages and floor pens. Poultry Science 61: 2166-2171.

50. **Petitte, J.N.,** R.O. Hawes, and R.W. Gerry. 1981. Control of flock uniformity of broiler breeder pullets through segregation according to body weight. Poultry Science 60: 2395-2400.

PATENTS

1. Pardue, S. L., **Petitte, J. N.,** D'Costa S and Song, Y. Methods for Gamete Production in Birds. Issued: February 17, 2004; US Patent # 6,691,638.

2. **Petitte, J. N.,** Ricks, C. A. and Spence, S. E. Gene transfer in poultry by introduction of embryo cells in ovo. Issued: February 4, 2003; US Patent # 6,515,199.

3. **Petitte, J. N.,** Ricks, C. A., Phelps, P. V. and Williams, C. Gene transfer in chickens by introduction of DNA into muscle in ovo. Issued: May 28, 2002; US Patent #6,395,961.

4. Pardue, S. L., **Petitte, J. N.,** and D'Costa, S. Methods for Gamete Production in Birds. Issued: March 12, 2002; US Patent # 6,354,242.

5. **Petitte, J. N.** and Chang, I. Method of Producing an Undifferentiated Avian Cell Culture using Avian Primordial Germ Cells. Issued: December 25, 2001; US Patent # 6,333,192.

6. **Petitte, J. N.** and Yang, Z. Veterinary Pharmaceutical Formulation Containing Avian Embryonic Stem cells. Issued: November 3, 1998; U.S. Patent # 5,830,510.

7. **Petitte, J.** and C. A. Ricks, Apparatus for Injecting Avian Embryo Muscle Tissue In Ovo, Issued: July 28, 1998, U.S. Patent #5,784,992.

8. **Petitte, J. N.** and Yang, Z. Avian Embryonic Stem Cells. Issued: August 12, 1997, U.S. Patent No. 5,656,479.

9. **Petitte, J. N.** and Yang, Z. Method of Producing an Avian Embryonic Stem Cell Culture and the Avian Embryonic Stem Cell Culture Produced by the Process. Issued: August 23, 1994, U.S. Patent No. 5,340,740.

10. **Petitte, J. N.**, Ricks, C., and Spence, S. Methods of Transferring DNA into birds by Somatic Cell Injection. U.S. Patent Pending. Filed January, 1992.

11. **Petitte, J. N.** and Ricks, C. Gene Transfer in Birds by Introduction of DNA into Muscle in Ovo. U.S. Patent Pending. Filed January, 1992.

BOOK CHAPTERS

1. **Petitte, J. N.**, 2004. Isolation and maintenance of avian ES Cells. Chapter 44, pp. 471-478 in: Handbook of Stem Cells, Vol. 1. Embryonic Stem Cells. R. Lanza, J. Gearhart, B. Hogan, D. Melton, R. Pedersen, J. Thomson and M. West, eds. Elsivier Academic Press, Burlington, MA.

2. **Petitte, J. N.** 2003. Strategies for the production of transgenic poultry. Chapter 33, pp. 665-684 in: Poultry Genetics, Breeding and Biotechnology. W. M. Muir and S. E. Aggrey, eds. CABI Publishing.

3. **Petitte, J. N.** and Mozdziak, P.E. 2002. Transgenic Poultry. Chapter 10 in Transgenic Animal Technology, A laboratory Handbook. C.A. Pinkert, Editor. Academic Press.

4. Rodriguez, G.C., D. Walmer, M. Cline, H. Krigham, R. Whitaker, P.D. Isner, B. Lessey, C. McMahon, J. Marks, **J. Petitte**, D. Carver, K. Anderson, A. Burchuck, J. Barnes, and C. Hughes, 2002. Part 4: Prevention and Screening; Chapter 23, "Biologic Effects of Progestins on Ovarian Epithelium: Cancer Prevention Through Apoptosis.", pp. 161-170, Ovarian Cancer, Edited by: I.J. Jacobs, J.H. Shepard, D.H. Oram, A.D. Blackett, D.M. Luesley, A. Berchuck, and C.H. Hudson, Oxford University Press

5. **Petitte, J. N.** and G. Davis. 1999. Breeding and Genetics. Chapter 11, In: The Ostrich: Biology, Production and Health, ed. D.C. Deeming, Wallingford, UK, CAB International.

6. **Petitte, J. N.**, S. D'Costa, and L., Karagenç. 1999. Understanding the Origin of Avian Primordial Germ Cells:Implications for Germ Cell Culture and Transgenesis in Poultry. In Transgenic Animals in Agriculture, eds. J.D. Murray, G.B. Anderson, A.M. Oberbauer, and M.M. McGloughlin. CAB International. P. 97-116.

7. **Petitte, J.N.**, J.M. Petitte and S. Scheideler. 1996. Determination of genetic diversity in commercial ratite stocks using multilocus DNA fingerprinting. Pages 69-77 in "Improving our understanding of ratites in a farming environment", ed. Deeming, ed., Ratite Conference: Banbury

8. **Petitte, J.N.**, C.L. Brazolot, M.E. Clark, G. Liu, A.M. Verrinder Gibbins, and R.J. Etches. 1993. Accessing the genome of the chicken using germline chimeras. In: Manipulation of the Avian Genome, R.J. Etches and A.M.V. Gibbins, eds., CRC, Ann Arbor.

9. Etches, R.J., J.N. Petitte, A.M. Verrinder Gibbins, C.L. Brazolot, and G. Liu. 1991. Production of chimeric chickens by blastodermal stem cell transfer and the prospects for gene manipulation. Chapter 22, pp 305-309 in: Avian Incubation Poultry Science Symposium 22, S.G. Tullett, ed. Butterworth-Heinemannm, London.

THESES SUPERVISED

1. Song, Y. 2003. Production of mixed-sex germline chimeras in the chicken. Ph.D Thesis, NC State University.

2. Borwornpinyo, S., 2000. Optimal hatchability of cultured chicken embryos from freshly laid eggs, M.S. Thesis, NC State University.

3. D'Costa, S., 1999. Characterization of turkey primordial germ cells and the production of interspecific embryonic chimeras. Ph.D. Thesis, NC State University.

4. Karagenc, L., 1998. Development of avian primordial germ cells in vivo and in vitro. Ph.D. Thesis, NC State University.

POSTDOCTORAL TRAINEES

Zengming Yang, 1991-1992
Carol Bolnet, 1992-1994
Guodong Liu, 1995
Levent Karagenc, 1999
Il-kuk Chang, 1999
Susan D'Costa, 2000-2001

FUNDING HISTORY (Total Amount Awarded: $2,821,649.00)

1. NCSU Faculty Research & Professional Development Fund: Title Evaluation of Biological Changes in Transgenic Chickens. Project Leaders: C. Ashwell, P.E. Mozdziak, **J. N. Petitte**, Amount: $15,000 Duration: 04/01/2004 - 03/31/2005.

2. Hubbard-ISA, Walpole, NH, a division of Merial. Title: A method for intra- and inter-species gamete production. Project Leaders: S. L. Pardue and **J. N. Petitte**, Amount: $955,595.00 Duration: 1/1/2003-8/31/2005

3. Duke University (Prime--National Institutes of Health) Title: Preclinical Evaluation of Intermediate Endpoints and Their Modulation by Chemopreventive Agents : D. Carver, K.E. Anderson, **J.N. Petitte**, G. Davis, H.J. Barnes. Amount: $379,705.00. Duration: 09/30/2000 - 08/31/2005.

4. Northwestern University (Prime--US Army-DOD) Title: Evaluation of Progestins and Vitamin D for the Chemoprevention of Ovarian Cancer. Project Leaders: K.E. Anderson, D. Carver, **J.N. Petitte**, G. Davis. Amount: $39,639.00. Duration: 10/01/2002 - 09/30/2004.

5. Hubbard-ISA, Walpole, NH, a division of Merial. Title: A method for intra- and inter-species gamete production. Project Leaders: S. L. Pardue and **J. N. Petitte**, Amount: $251,347. Duration: 4/15/00-12/31/02

6. Duke University: Title: Ovarian Cancer in Chickens. Project Leaders: **J.N. Petitte**, K.E. Anderson, and D. Carver, Amount: $107,595. Duration: 10/31/99-10/31/00.

7. Duke University: Title: Preclinical Evaluation of Intermediate Endpoints and Their Modulation by Chemoprevention Agents. Project Leaders: D. K. Carver, K. E. Anderson, **J. N. Petitte**, G. S. Davis, H. J. Barnes. Amount $399,629. Duration: 10/01/00-09/03/003.

8. Origen Therapuetics, Inc. Title: Avian Embryonic Stem Cells. Project Leader: **J.N. Petitte**, Amount: $346,119. Duration: 7/14/97-6/30/01.

9. NSF, Title: Analysis of avian primordial germ cell development in vitro and in vivo. Project Leader, **J.N. Petitte**, Amount: $200,000 9/15/96 - 8/31/99.

10. USDA-NRI, Title: Avian Embryonic Stem Cells, Project Leader: **J.N. Petitte**, Amount: $165,000, Duration: 9/1/94 - 8/31/97.

11. North Carolina Biotechnology Center-ARIG, Sex-specific DNA in Ratites, Project Leader: **J.N. Petitte**, Duration: 7/1/94-12/31/96, $39,000.

12. North Carolina Biotechnology Center-ARIG, "Establishment of Avian Blastodermal Cell Culture for the Development of Transgenic Poultry", Project Leader: **J.N. Petitte**, Duration: 8/1/91-1/31/93, $40,000.

13. Southeastern Poultry and Egg Association "Improving Embryonic Viability of Stored Turkey Eggs. I. Effects of Preincubational Embryonic Development on Hatchability" Project Leaders: V. Christensen, M. Wineland , and **J.N. Petitte**, Amount: $29,000 , Duration: 1/1/93-12/31/93.

14. USDA,-NRI, "Avian Embryonic Stem Cell Lines and the Development of Transgenic Poultry" Project Leader: **J.N. Petitte**, Amount: $50,000. Duration: 9/1/91 - 8/31/93,

15. USDA/BARD, "The Study of Primordial Germ Cell Development as a Tool for Gene Transfer in chickens" Project Leader: **J.N. Petitte**, H. Eyal-Giladi and M. Ginsburg, The Hebrew University, Amount $22,000 Duration: 10/1/92 - 9/30/95.

16. Faculty Research and Professional Development Fund, "State-Specific Antigen Expression During Development of the Early Chick Embryo", Project Leader: **J.N. Petitte**, Duration: 7/1/91-9/1/91, $3,500.

17. North Carolina Biotechnology Center, Educational Enhancement Grant, "Faculty Training in cDNA cloning and Gene Expression", Project Leader: **J.N. Petitte**, Duration: 7/1/91-9/1/91, $1,500.

18. Embrex, Inc., "Automated, Somatic Cell Gene Targeting Transfer in Poultry", Project Leader: **J.N. Petitte**, Amount $152,735, Duration: 1/1/92-12/31/93.

INVITED PRESENTATIONS

1. PSA 2005 Annual Meeting, Ancillary Scientists Program, "Avian Germplasm Preservation: stem cells or PGCs?" July 30, 2005, Auburn.

2. 54th National Breeders Roundtable 2005, "Transgenic Technologies: Current Successes and Future Directions", May 5, 2005, St. Louis

3. Comparative Biomedical Sciences, College of Veterinary Medicine, "Avian Primordial Germ Cells at the Interface of Poultry Biotechnology", January 21, 2004

4. Graduate School of Bioagricultural Sciences, University of Nagoya, Japan, "Avian Embryonic Stem Cells and Transgenic Poultry", July 2001.

5. Department of Animal Science, Genetic Group Seminar, NC State University, "Avian Embryonic Stem Cells and Transgenic Poultry", April 2001.

6. USDA-ARS, Growth Biology Laboratory, Beltsville Maryland, "Avian Embryonic Chimeras and Transgenic Poultry", December 2000.

7. Department of Zoology, NC State University, "The Origin of Avian Primordial Germ Cells in the Pre-streak Embryo", March 2000.

8. Department of Poultry Science, University of Arkansas, "Biotechnology in Agriculture: Applications in the Poultry Industry", November 2000.

9. Department of Poultry Science, University of Arkansas, "Avian Embryonic Chimeras and Transgenic Poultry", November 2000.

10. World Poultry Congress, "Role of Growth Factors in Early Embryonic Development", Montreal, August 2000.

11. 12th Symposium on Current Problems in Avian Genetics, "Growth Factors in Avian Primordial Germ Cell Development", Prague, Czech Republic, September 1997.

12. Transgenic Animals in Agriculture, "Culture of Avian PGCs for Transgenesis in Poultry", Tahoe, CA, August 1997.

13. Poultry Science Association Annual Meetings, Ancillary Scientist Symposium Genetic Selection Strategies for the Future, "Primordial Germ Cells Manipulation", July 1996

14. Improving Our Understanding of Ratites in a Farming Environment, "Determination of Genetic Diversity in Commercial Ratite Stocks using Multilocus DNA Fingerprinting", Manchester England, March 1996.

15. North Carolina Emu Seminar and Trade Show, "DNA Science and the Ratite Industry: Current and Future Applications", Rockingham College, NC, November 1996.

16. 45th Annual National Breeders Roundtable, "Current Technologies for Transgenic Poultry", St. Louis, MO, May 1996.

17. Third Annual Oklahoma Ratite Seminar, "Selection of Replacement Stock", Oklahoma City, October 1995.

18. American Ostrich Breeders Association Annual Meeting, "Information Magnagement for the Genetic Improvement of Ratites", January 1995

19. American Ostrich Breeders Association Annual Meeting, "Selection of Replacement Stock", January 1995.

20. Department of Animal Science, University of Delaware, "Avian Embryonic Stem Cells", November 1995.

21. North Carolina Ostrich Breeders Association, "Selection of Replacement Stock", November 1995.

22. American Society of Zoology, "Growth Factors in Early Embryonic Development", December 1995.

23. North Carolina Ostrich Breeders Association, "Application of DNA Science to the Ratite Industry", November 1994

24. Department of Biology, Pembrooke State University, "Progress Towards Manipulation of the Avian Genome", February 1994

25. Duke Institute for Learning in Retirement, "Application of biotechnology to the Poultry Industry", February 1994.

26. Department of Molecular and Cellular Biology, Siblermen Institute of Life Sciences, The Hebrew University of Jerusalem, "Prospects for manipulation of the avian embryo using early embryonic chimeras", May 1994.

27. Animal Biotechnology Seminar Program, University of Minnesota, "Progress towards the development of transgenic poultry using avian embryonic stem cells", May 1993.

28. NCSU Biotechnology Program Retreat, NC Biotechnology Center, "Accessing the avian genome using germline chimeras", February 1993.

29. European Molecular Biology Laboratory, Heidleburg, Germany, "Development of avian embryonic chimeras and embryonic stem cells", May 1992.

30. Institute of Cellular and Molecular Embryology, CNRA College of France, Nogent sur Marne, "Progress towards the development of avian embryonic stem cells", May 1992

31. Triangle Transgenics Group, "Accessing the avian genome using blastodermal chimeras", November 1991.

32. Department of Poultry Science, University of Georgia, "Production of germ line chimeras in the chicken and the development of transgenic poultry", November 1991.

33. Department of Microbiology and Immunology, School of Medicine, East Carolina University, "Development of germline chimeras and prospects for the manipulation for the avian genome. November 1991.

34. USDA Beltsville, MD, "Development of somatic and germ line chimeras in the chicken", April 1991.

35. Keystone Symposia on Molecular and Cellular Biology: Manipulation of the Avian Genome, "Accessing the avian genome using germline chimeras", March 1991.

RESEARCH ABSTRACTS

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2. Pophal, S. P. E. Mozdziak, S. Borwornpinyo, and **J. N. Petitte**, 2004. Transgenic Chickens Expressing Beta-galactosidase Hydrolyze Lactose In The Intestine. Poultry Sci. (Suppl. 1) 83: 2810.

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COMMITTEE ACTIVITIES

Departmental:
Graduate Studies Committee
Equipment Prioritization Committee
Safety & Hazardous Chemicals Committee
Seminar Committee
Computer Committee (Chairman, 1997-2001)

College:
CALS Research Committee: Chair of EPA promotions sub-committee.
CALS Academic Computing and Advisory Committee
CALS Safety Committee

Regional:
USDA Multistate Regional Project NC-168 Genetic Improvement of Poultry
USDA Multistate Regional Project NRSP-8 National Animal Genome Research Program

National:
External Program Committee for the Transgenic Animal Conference
Poultry Science Association:
Ancillary Scientist Liaison Committee (Chairman)
Poultry Science Research Award Committee
USDA NRI: Review Panel for Competitive Grants Program
Section: Enhancing Animal Reproductive Efficiency
USDA IFAFS: Review Panel for Competitive Grants Program
Section: Animal Genomics

***Ad Hoc* Grant Reviews:**
USDA National Initiative Competitive Grants Program

USDA SBIR Program
National Science Foundation
USDA BARD

***Ad Hoc* Journal Reviews:**
Biology of Reproduction
Transgenic Research
International Journal of Developmental Biology
Poultry Science
Journal of Veterinary Diagnostic Investigation
Journal of Heredity
Biotechniques
British Poultry Science
Journal of Experimental Zoology
Nature Biotechnology